

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF ELECTION
 (PCT Rule 61.2)

To:

United States Patent and Trademark
 Office
 (Box PCT)
 Crystal Plaza 2
 Washington, DC 20231
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 October 1996 (23.10.96)	
International application No. PCT/GB96/00553	Applicant's or agent's file reference 27.42.62655/002
International filing date (day/month/year) 08 March 1996 (08.03.96)	Priority date (day/month/year) 10 March 1995 (10.03.95)
Applicant GIERSKCKY, Karl, Erik et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

09 October 1996 (09.10.96)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Carlos Roy Telephone No.: (41-22) 730.91.11
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PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference

(if desired) (12 characters maximum) 27.42.62655/002

Box No. I TITLE OF INVENTION

Derivatives of 5-amino levulinic acids

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

The Norwegian Radium Hospital Research Foundation,
Montebello
0310 Oslo 3
Norway

This person is also inventor.

Telephone No.

Faximile No.

Teleprinter No.

State (i.e. country) of nationality:

NO

State (i.e. country) of residence:

NO

This person is applicant for the purposes of:

all designated States

all designated States except the United States of America

the United States of America only

the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

GIERSKCKY, Karl Erik
c/o The Norwegian Radium Hospital
Montebello
0310 Oslo
Norway

This person is:

applicant only

applicant and inventor

inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality:

NO

State (i.e. country) of residence:

NO

This person is applicant for the purposes of:

all designated States

all designated States except the United States of America

the United States of America only

the States indicated in the Supplemental Box

Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

agent

common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

DZIEGLEWSKA, Hanna Eva
Frank B. Dehn & Co
Imperial House
15-19 Kingsway
London WC2B 6UZ

Telephone No.

0171 836 6193

Faximile No.

0171 240 0776

Teleprinter No.

261620

Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

*If none of the following sub-boxes is used, this sheet is not to be included in the request.*Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

MOAN, Johan
c/o Institute of Cancer Research
Montebello
0310 Oslo
Norway

This person is:

- applicant only
 applicant and inventor
 inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

NO

State (i.e. country) of residence:

NO

This person is applicant for the purposes of:

 all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental BoxName and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

PENG, Qian
c/o Institute of Cancer Research
Montebello
0310 Oslo
Norway

This person is:

- applicant only
 applicant and inventor
 inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

CN

State (i.e. country) of residence:

NO

This person is applicant for the purposes of:

 all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental BoxName and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

STEEN, Harald
c/o Institute of Cancer Research
Montebello
0310 Oslo
Norway

This person is:

- applicant only
 applicant and inventor
 inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

NO

State (i.e. country) of residence:

NO

This person is applicant for the purposes of:

 all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental BoxName and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

WARLOE, Trond
c/o The Norwegian Radium Hospital
Montebello
0310 Oslo
Norway

This person is:

- applicant only
 applicant and inventor
 inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

NO

State (i.e. country) of residence:

NO

This person is applicant for the purposes of:

 all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

*If none of the following sub-boxes is used, this sheet is not to be included in the request.*Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BJORSETH, ALF
 c/o Norwegian Radium Hospital Research
 Foundation
 Montebello
 0310 Oslo 3
 Norway

This person is:

- applicant only
 applicant and inventor
 inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

NO

State (i.e. country) of residence:

NO

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental BoxName and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DZIEGLEWSKA, Hanna Eva
 c/o Frank B Dehn & Co
 Imperial House
 15-19 Kingsway
 London WC2B 6UZ

This person is:

- applicant only
 applicant and inventor
 inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

GB

State (i.e. country) of residence:

GB

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental BoxName and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:

- applicant only
 applicant and inventor
 inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

State (i.e. country) of residence:

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental BoxName and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:

- applicant only
 applicant and inventor
 inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

State (i.e. country) of residence:

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

**Kyrgyzstan, Moldova, Armenia

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

Regional Patent

- AP ARIPO Patent: KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- EA Eurasian Patent: AZ Azerbaijan, BY Belarus, KZ Kazakstan, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT **see above
- EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT - FI Finland
- OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | |
|--|--|
| <input checked="" type="checkbox"/> AL Albania | <input checked="" type="checkbox"/> MD Republic of Moldova |
| <input checked="" type="checkbox"/> AM Armenia | <input checked="" type="checkbox"/> MG Madagascar |
| <input checked="" type="checkbox"/> AT Austria | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia |
| <input checked="" type="checkbox"/> AU Australia | |
| <input checked="" type="checkbox"/> AZ Azerbaijan | <input checked="" type="checkbox"/> MN Mongolia |
| <input checked="" type="checkbox"/> BB Barbados | <input checked="" type="checkbox"/> MW Malawi |
| <input checked="" type="checkbox"/> BG Bulgaria | <input checked="" type="checkbox"/> MX Mexico |
| <input checked="" type="checkbox"/> BR Brazil | <input checked="" type="checkbox"/> NO Norway |
| <input checked="" type="checkbox"/> BY Belarus | <input checked="" type="checkbox"/> NZ New Zealand |
| <input checked="" type="checkbox"/> CA Canada | <input checked="" type="checkbox"/> PL Poland |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein | <input checked="" type="checkbox"/> PT Portugal |
| <input checked="" type="checkbox"/> CN China | <input checked="" type="checkbox"/> RO Romania |
| <input checked="" type="checkbox"/> CZ Czech Republic | <input checked="" type="checkbox"/> RU Russian Federation |
| <input checked="" type="checkbox"/> DE Germany | <input checked="" type="checkbox"/> SD Sudan |
| <input checked="" type="checkbox"/> DK Denmark | <input checked="" type="checkbox"/> SE Sweden |
| <input checked="" type="checkbox"/> EE Estonia | <input checked="" type="checkbox"/> SG Singapore |
| <input checked="" type="checkbox"/> ES Spain | <input checked="" type="checkbox"/> SI Slovenia |
| <input checked="" type="checkbox"/> FI Finland | <input checked="" type="checkbox"/> SK Slovakia |
| <input checked="" type="checkbox"/> GB United Kingdom | <input checked="" type="checkbox"/> TJ Tajikistan |
| <input checked="" type="checkbox"/> GE Georgia | <input checked="" type="checkbox"/> TM Turkmenistan |
| <input checked="" type="checkbox"/> HU Hungary | <input checked="" type="checkbox"/> TR Turkey |
| <input checked="" type="checkbox"/> IS Iceland | <input checked="" type="checkbox"/> TT Trinidad and Tobago |
| <input checked="" type="checkbox"/> JP Japan | <input checked="" type="checkbox"/> UA Ukraine |
| <input checked="" type="checkbox"/> KE Kenya | <input checked="" type="checkbox"/> UG Uganda |
| <input checked="" type="checkbox"/> KG Kyrgyzstan | <input checked="" type="checkbox"/> US United States of America |
| <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea | |
| <input checked="" type="checkbox"/> KR Republic of Korea | <input checked="" type="checkbox"/> UZ Uzbekistan |
| <input checked="" type="checkbox"/> KZ Kazakstan | <input checked="" type="checkbox"/> VN Viet Nam |
| <input checked="" type="checkbox"/> LK Sri Lanka | |
| <input checked="" type="checkbox"/> LR Liberia | |
| <input checked="" type="checkbox"/> LS Lesotho | |
| <input checked="" type="checkbox"/> LT Lithuania | |
| <input checked="" type="checkbox"/> LU Luxembourg | |
| <input checked="" type="checkbox"/> LV Latvia | |

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- | |
|--------------------------------|
| <input type="checkbox"/> |

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.
 The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

Supplemental Box*If the Supplemental Box is not used, this sheet need not be included in the request.***Use this box in the following cases:****1. If, in any of the Boxes, the space is insufficient to furnish all the information:****in particular:**(i) **if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available:**(ii) **if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked:**(iii) **if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:**(iv) **if, in addition to the agent(s) indicated in Box No. IV, there are further agents:**(v) **if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part":**(vi) **if there are more than three earlier applications whose priority is claimed:****2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty:****in such case, write "Continuation of Box No. ..." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;****in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III;****in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, European or OAPI patent) for the purposes of which the named person is applicant;****in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, European or OAPI patent) for the purposes of which the named person is inventor;****in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;****in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;****in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;****in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.****Continuation of Box No. III**

Dzieglewska, Hanna Eva is applicant in respect of the GB designation (national application and regional application via European Patent Office).

Continuation of Box No. IV

The following, also of Frank B. Dehn & Co., are also appointed as agents for the applicants:

WATKINS, Arnold J; LEALE, Robin G; HILDYARD, Dr E Martin; SKAILES, Humphrey J; WOODMAN, Derek; TOMLINSON, Kerry J; BUTLER, Michael J; PETT, Christopher P; COCKBAIN, Julian R M; DAVIES, Christopher R; PIESOLD, Alexander J; MATTHEWS, Derek P; CALAMITA, Roberto; DAVIES, Sophie J; MARSDEN, John C; HAGUE, Alison J; LECKEY, David H; TOWLER, Philip D; HUGHES, Andrea M; REED, Michael A and PRICE, Anthony S all having the same address as set forth in Box IV.

Box No. VI PRIORITY CLAIMFurther priority claims are indicated in the Supplemental Box

The priority of the following earlier application(s) is hereby claimed:

Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
item (1) GB	10 March 1995 (10.03.95)	9504948.2	
item (2) GB	18 December 1995 (18.12.95)	9525822.4	
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required): The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): (1) + (2)**Box No. VII INTERNATIONAL SEARCHING AUTHORITY****Choice of International Searching Authority (ISA)** (If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used): ISA / _____**Earlier search** Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.

Country (or regional Office): Date (day/month/year): Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	: 6	sheets
2. description	: 35	sheets
3. claims	: 4	sheets
4. abstract	: 1	sheets
5. drawings	: 23	sheets
Total	:	69 sheets

This international application is accompanied by the item(s) marked below:

- | | |
|---|--|
| 1. <input type="checkbox"/> separate signed power of attorney | 5. <input checked="" type="checkbox"/> fee calculation sheet |
| 2. <input checked="" type="checkbox"/> copy of general power of attorney | 6. <input type="checkbox"/> separate indications concerning deposited microorganisms |
| 3. <input type="checkbox"/> statement explaining lack of signature | 7. <input type="checkbox"/> nucleotide and/or amino acid sequence listing (diskette) |
| 4. <input type="checkbox"/> priority document(s) identified in Box No. VI as item(s): | 8. <input checked="" type="checkbox"/> other (specify) Form 24/77 |

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Hanna Dzieglewska

(Agent for the applicants)

For receiving Office use only

1. Date of actual receipt of the purported international application:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA /

2. Drawings:

 received: not received:6. Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

See Notes to the request form

PCT

FEE CALCULATION SHEET Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference 27.42.62655/002

Applicant

The Norwegian Radium Hospital REsearch Foundation

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE GBP 55 T
2. SEARCH FEE GBP 1104 S

International search to be carried out by _____

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 69 sheets.

$$\begin{array}{l} \text{first 30 sheets} \quad \text{GBP } 430 \quad b_1 \\ \underline{39} \quad \times \quad \underline{8} \quad = \quad \underline{\text{GBP } 312} \quad b_2 \\ \text{remaining sheets} \quad \text{additional amount} \end{array}$$

Add amounts entered at b₁ and b₂ and enter total at B GBP 742 B

Designation Fees

The international application contains _____ designations.

$$\begin{array}{l} 11 \quad \times \quad \text{GBP } 104 \quad = \quad \text{GBP } 1140 \quad D \\ \text{number of designation fees} \quad \text{amount of designation fee} \\ \text{payable (maximum 11)} \end{array}$$

Add amounts entered at B and D and enter total at I GBP 1882 I
(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT

GBP 44 P

5. TOTAL FEES PAYABLE

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

GBP 3085
TOTAL

The designation fees are not paid at this time.

MODE OF PAYMENT

authorization to charge deposit account (see below)
 cheque
 postal money order

bank draft
 cash
 revenue stamps

coupons
 other (specify): _____

DEPOSIT ACCOUNT AUTHORIZATION (this mode of payment may not be available at all receiving Offices)

The RO/ is hereby authorized to charge the total fees indicated above to my deposit account.

- is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.
 is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

Deposit Account Number

Date (day/month/year)

Signature

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year) 21 March 1997 (21.03.97)	To: DZIEGLEWSKA, Hanna, Eva Frank B. Dehn & Co. 179 Queen Victoria Street London EC4V 4EL ROYAUME-UNI
Applicant's or agent's file reference 27.42.62655/002	IMPORTANT NOTIFICATION
International application No. PCT/GB96/00553	International filing date (day/month/year) 08 March 1996 (08.03.96)

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address THE NORWEGIAN RADIUM HOSPITAL RESEARCH FOUNDATION Montebello N-0310 Oslo 3 NO	State of Nationality NO	State of Residence NO
Telephone No.		
Facsimile No.		
Teleprinter No.		

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address PHOTOCURE AS Norveien 7 Montebello N-0310 Oslo NO	State of Nationality NO	State of Residence NO
Telephone No.		
Facsimile No.		
Teleprinter No.		

3. Further observations, if necessary:

4. A copy of this notification has been sent to:	
--	--

the receiving Office the designated Offices concerned
 the International Searching Authority the elected Offices concerned
 the International Preliminary Examining Authority other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Carlos Roy Telephone No.: (41-22) 730.91.11
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

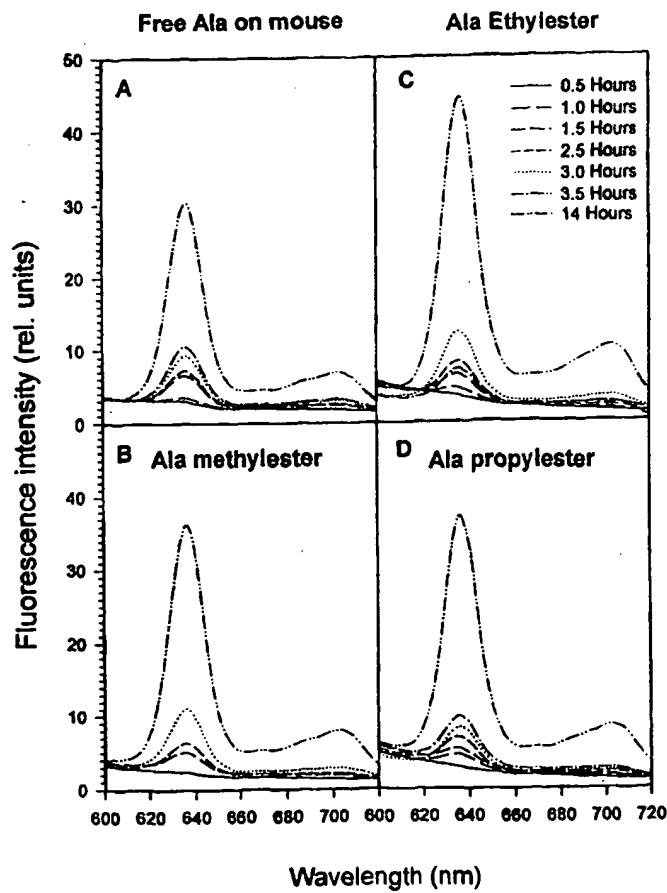
(51) International Patent Classification ⁶ : C07C 229/22, 227/18, A61K 31/195	A1	(11) International Publication Number: WO 96/28412 (43) International Publication Date: 19 September 1996 (19.09.96)
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(21) International Application Number: PCT/GB96/00553	(74) Agents: DZIEGLEWSKA, Hanna, Eva et al.; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).
(22) International Filing Date: 8 March 1996 (08.03.96)	
(30) Priority Data: 9504948.2 10 March 1995 (10.03.95) GB 9525822.4 18 December 1995 (18.12.95) GB	(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(71) Applicant (for all designated States except US): THE NORWEGIAN RADIUM HOSPITAL RESEARCH FOUNDATION [NO/NO]; Montebello, N-0310 Oslo 3 (NO).	
(71) Applicant (for GB only): DZIEGLEWSKA, Hanna, Eva [GB/GB]; Frank B. Dehn & Co., Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).	
(72) Inventors; and	
(75) Inventors/Applicants (for US only): GIERSKCKY, Karl, Erik [NO/NO]; The Norwegian Radium Hospital, Montebello, N-0310 Oslo (NO). MOAN, Johan [NO/NO]; (NO). PENG, Qian [CN/NO]; (NO). STEEN, Harald [NO/NO]; Institute of Cancer Research, Montebello, N-0310 Oslo (NO). WARLOE, Trond [NO/NO]; The Norwegian Radium Hospital, Montebello, N-0310 Oslo (NO). BJORSETH, Alf [NO/NO]; Norwegian Radium Hospital Research Foundation, Montebello, N-0310 Oslo 3 (NO).	Published With international search report.

(54) Title: ESTERS OF 5-AMINOLEVULINIC ACID AS PHOTOSENSITIZING AGENTS IN PHOTOCHEMOTHERAPY

(57) Abstract

The present invention relates to compounds being esters of 5-aminolevulinic acids or pharmaceutically acceptable salts thereof, including compounds of formula (I) $R_2^2N\text{-CH}_2\text{COCH}_2\text{-CH}_2\text{CO-OR}^1$, (wherein R^1 may represent alkyl optionally substituted by hydroxy, alkoxy, acyloxy, alkoxy carbonyloxy, amino, aryl, oxo or fluoro groups and optionally interrupted by oxygen, nitrogen, sulphur or phosphorus atoms; and R^2 represents a hydrogen atom or a group R^1 , and both R^2 groups may be the identical or different), and their use in diagnosis and phototherapy of disorders or abnormalities of external or internal surfaces of the body, and products and kits for performing the invention.



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88 Rec'd PCT/PTO 10 SEP 1997

- 1 -

ESTERS OF 5-AMINOLEVULINIC ACID AS PHOTOSENSITIZING AGENTS IN PHOTOCHEMOTHERAPY

The present invention relates to novel derivatives of 5-aminolevulinic acid (ALA) and in particular to novel esters of ALA useful as photosensitizing agents in photochemotherapy.

Photochemotherapy, or photodynamic therapy (PDT) as it is also known, is a recently up-coming technique for the treatment of various abnormalities or disorders of the skin or other epithelial organs or mucosa, especially cancers or pre-cancerous lesions, as well as certain non-malignant lesions for example skin complaints such as psoriasis. Photochemotherapy involves the application of photosensitizing (photochemotherapeutic) agents to the affected area of the body, followed by exposure to photoactivating light in order to activate the photosensitizing agents and convert them into cytotoxic form, whereby the affected cells are killed or their proliferative potential diminished.

A range of photosensitizing agents are known, including notably the psoralens, the porphyrins, the chlorins and the phthalocyanins. Such drugs become toxic when exposed to light.

Photosensitizing drugs may exert their effects by a variety of mechanisms, directly or indirectly. Thus for example, certain photosensitizers become directly toxic when activated by light, whereas others act to generate toxic species, e.g. oxidising agents such as singlet oxygen or other oxygen-derived free radicals, which are extremely destructive to cellular material and biomolecules such as lipids, proteins and nucleic acids. Psoralens are an example of directly acting photosensitizers; upon exposure to light they form adducts and cross-links between the two strands of DNA

- 2 -

molecules, thereby inhibiting DNA synthesis. The unfortunate risk with this therapy is that unwanted mutagenic and carcinogenic side effects may occur.

This disadvantage may be avoided by selecting photosensitizers with an alternative, indirect mode of action. For example porphyrins, which act indirectly by generation of toxic oxygen species, have no mutagenic side effects and represent more favourable candidates for photochemotherapy. Porphyrins are naturally occurring precursors in the synthesis of heme. In particular, heme is produced when iron (Fe^{3+}) is incorporated in protoporphyrin IX (Pp) by the action of the enzyme ferrochelatase. Pp is an extremely potent photosensitizer, whereas heme has no photosensitizing effect.

One such porphyrin-based drug, Photofrin, has recently been approved as a photosensitizer in the therapy of certain cancers. The main disadvantage is that since it must be administered parenterally, generally intravenously, cause photosensitization of the skin which may last for several weeks following i.v. injection. Photofrin consists of large oligomers of porphyrin and it does not readily penetrate the skin when applied topically. Similar problems exist with other porphyrin-based photosensitizers such as the so-called "hematoporphyrin derivative" (Hpd) which has also been reported for use in cancer photochemotherapy (see for example S. Dougherty. J. Natl. Cancer Inst., 1974, 52; 1333; Kelly and Snell, J. Urol, 1976, 115 : 150). Hpd is a complex mixture obtained by treating haematoporphyrin with acetic and sulphuric acids, after which the acetylated product is dissolved with alkali.

To overcome these problems, precursors of Pp have been investigated for photochemotherapeutic potential. In particular the Pp precursor 5-aminolevulinic acid (ALA) has been investigated as a photochemotherapeutic agent for certain skin cancers. ALA, which is formed

- 3 -

from succinyl CoA and glycine in the first step of heme synthesis, is to a limited extent able to penetrate the skin and lead to a localised build-up of Pp; since the action of ferrochelatase (the metallating enzyme) is the rate limiting step in heme synthesis, an excess of ALA leads to accumulation of Pp, the photosensitizing agent. Thus, by applying ALA topically to skin tumours, and then after several hours exposing the tumours to light, a beneficial photochemotherapy effect may be obtained (see for example WO91/01727). Since the skin covering basilomas and squamous cell carcinomas is more readily penetrated by ALA than healthy skin, and since the concentration of ferrochelatase is low in skin tumours, it has been found that topical application of ALA leads to a selectively enhanced production of Pp in tumours.

However, whilst the use of ALA represents a significant advance in the art, photochemotherapy with ALA is not always entirely satisfactory. ALA is not able to penetrate all tumours and other tissues with sufficient efficacy to enable treatment of a wide range of tumours or other conditions and ALA also tends to be unstable in pharmaceutical formulations. A need therefore exists for improved photochemotherapy agents.

The present invention addresses this need and in particular aims to provide novel photochemotherapy agents which are better able to penetrate the tumour or other abnormality, and which have an enhanced photochemotherapy effect over those described in the prior art.

In one aspect, the present invention thus provides compounds being esters of 5-aminolevulinic acids or pharmaceutically acceptable salts thereof.

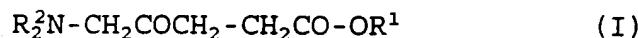
In the esters of the invention the 5-amino group may be substituted or unsubstituted, the latter case being the ALA esters.

- 4 -

In another aspect the present invention also provides such compounds, or salts thereof, for use in photochemotherapy or diagnosis.

More particularly, the compounds of the invention are esters of 5-aminolevulinic acids with optionally substituted alkanols, ie. alkyl esters or substituted alkyl esters.

Alternatively viewed, the invention can therefore be seen to provide compounds of formula I,



(wherein R¹ may represent alkyl optionally substituted by hydroxy, alkoxy, acyloxy, alkoxy carbonyloxy, amino, aryl, oxo or fluoro groups and optionally interrupted by oxygen, nitrogen, sulphur or phosphorus atoms; and R², each of which may be the same or different, represents a hydrogen atom or a group R¹) and salts thereof.

The substituted alkyl R¹ groups may be mono or poly-substituted. Thus suitable R¹ groups include for example unsubstituted alkyl, alkoxyalkyl, hydroxyalkoxyalkyl, polyhydroxyalkyl, hydroxy poly alkyleneoxyalkyl and the like. The term "acyl" as used herein includes both carboxylate and carbonate groups, thus, acyloxy substituted alkyl groups include for example alkylcarbonyloxy alkyl. In such groups any alkylene moieties preferably have carbon atom contents defined for alkyl groups below. Preferred aryl groups include phenyl and monocyclic 5-7 membered heteroaromatics, especially phenyl and such groups may themselves optionally be substituted.

Representative substituted alkyl groups R¹ include alkoxy methyl, alkoxy ethyl and alkoxy propyl groups or acyloxy methyl, acyloxy ethyl and acyloxy propyl groups eg. pivaloyloxy methyl.

Preferred compounds of the invention, include those wherein R¹ represents an unsubstituted alkyl group and/or

- 5 -

each R² represents a hydrogen atom.

As used herein, the term "alkyl" includes any long or short chain, straight-chained or branched aliphatic saturated or unsaturated hydrocarbon group. The unsaturated alkyl groups may be mono- or polyunsaturated and include both alkenyl and alkynyl groups. Such groups may contain up to 40 carbon atoms. However, alkyl groups containing up to 10 eg. 8, more preferably up to 6, and especially preferably up to 4 carbon atoms are preferred.

Particular mention may be made of ALA-methylester, ALA-ethylester, ALA-propylester, ALA-hexylester, ALA-heptylester and ALA-octylester and salts thereof, which represent preferred compounds of the invention.

The compounds of the invention may be prepared using standard processes and procedures well-known in the art for derivatization of multi-functional compounds, and especially esterification. As known in the art, such esterification of compounds may involve protection and deprotection of appropriate groups such that only the required groups remain active and take part in the reaction under the conditions of the esterification. Thus for example the substituents of substituted alkanols used to prepare the esters may be protected during esterification. Similarly the NR₂² group on the compound contributing this group to compounds of formula I may be protected during the reaction and deprotected thereafter. Such protection/deprotection procedures are well known in the art for the preparation of derivatives, and in particular, esters of well known amino-acids, see for example Mcomie in "Protective Groups in Organic Chemistry", Plenum, 1973 and T.W. Greene in "Protective Groups in Organic Chemistry", Wiley-Interscience, 1981.

In a further aspect, the present invention thus provides a process for preparing the compounds of the invention, comprising forming an ester of the carboxy

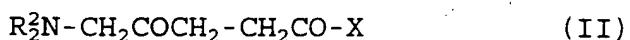
- 6 -

group of a 5-aminolevulinic acid.

The invention can thus be seen to provide a process for preparing the compounds of the invention, comprising reacting a 5-aminolevulinic acid, or an esterifiable derivative thereof, with an alkanol or an ester-forming derivative thereof.

More particularly, this aspect of the invention provides a process for preparing compounds of formula I, which process comprises at least one of the following steps:

(a) reacting a compound of formula II



(wherein X represents a leaving group, for example a hydroxyl group, a halogen atom or alkoxy group or COX represents an acid anhydride group and R² is as hereinbefore defined)

with a compound of formula III



(wherein R¹ is as hereinbefore defined); and

(b) converting a compound of formula I into a pharmaceutically acceptable salt thereof.

The reaction of step (a) may conveniently be carried out in a solvent or mixture of solvents such as water, acetone, diethylether, methylformamide, tetrahydrofuran etc. at temperatures up to the boiling point of the mixture, preferably at ambient temperatures.

The conditions of the esterification reactions will depend of the alcohol used and the conditions may be chosen such that maximum yield of the ester is obtained. Since the esterification reactions are reversible

- 7 -

equilibrium reactions, reaction conditions may be selected in such a way that maximum yield of the ester product is obtained. Such conditions may be obtained by selecting a solvent which is capable of removing the water formed in a typical esterification reaction by forming an azeotrope with water. Such solvents are exemplified by aromatic hydrocarbons or others capable of forming azeotropes with water, e.g. some chlorinated hydrocarbons such as chloroform. For the formation of the lower esters of 5-ALA the equilibrium reaction may be driven to the ester side by using a large excess of the alcohol. With other esters the equilibrium may be driven towards the ester product by using a large excess of the acid.

Esterification reactions are well-known in the art for example, as described by Saul Patai in "The chemistry of the carboxylic acids and esters", (Ch. 11, p. 505, Interscience 1969) and Houben Weyl, (Methoden der Organische Chemie, Band E5, "Carbonsäuren und carbonsäuren-derivate", p. 504, Georg Thieme Verlag, 1985). The formation of derivatives of amino-acids are described in Band XI/2 of the same series, (Houben Weyl, Methoden der Organische Chemie, Band XI/2, "Stickstoffverbindungen", p. 269, Georg Thieme Verlag, 1958).

The reaction will conveniently be carried out in the presence of a catalyst, e.g. an inorganic or organic acid or an acid binding agent such as a base.

The compounds used as starting materials are known from the literature, and in many cases commercially available, or may be obtained using methods known per se. ALA, for example, is available from Sigma or from Photocure, Oslo, Norway.

As mentioned above, the compounds of the invention may take the form of pharmaceutically acceptable salts. Such salts preferably are acid addition salts with physiologically acceptable organic or inorganic acids.

- 8 -

Suitable acids include, for example, hydrochloric, hydrobromic, sulphuric, phosphoric, acetic, lactic, citric, tartaric, succinic, maleic, fumaric and ascorbic acids. Procedures for salt formation are conventional in the art.

As mentioned above, the compounds of the invention and their salts have valuable pharmacological properties, namely a photosensitizing agent which renders them useful as photochemotherapeutic agents.

Like ALA, the compounds exert their effects by enhancing production of Pp; upon delivery to the desired site of action hydrolytic enzymes such as esterases present in the target cells break down the esters into the parent ALA, which then enters the haem synthesis pathway and leads to a build-up of Pp. However, the compounds of the invention have a number of advantages over ALA itself. Firstly, the novel compounds are better able to penetrate skin and other tissues as compared with ALA; the penetration is both deeper and faster. This is an important advantage, especially for topical administration. Secondly, the esters have surprisingly been found to be better enhancers of Pp production than ALA; Pp production levels following administration of the ALA esters are higher than with ALA alone. Thirdly, the compounds of the invention demonstrate improved selectivity for the target tissue to be treated, namely the Pp production-enhancing effect is localised to the desired target lesion and does not spread to the surrounding tissues. This is especially evident with tumours. Finally, the novel compounds appear to localise better to the target tissue upon administration. This is especially important for systemic application, since it means that undesirable photosensitization effects, as reported in the literature for other porphyrin-based photosensitizers, may be reduced or avoided.

A further aspect of the present invention

- 9 -

accordingly provides a pharmaceutical composition comprising a compound of the invention, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutical carrier or excipient.

In a still further aspect, there is also provided the use of a compound of the invention, or a pharmaceutically acceptable salt thereof, for the preparation of a therapeutic agent for use in photochemotherapy, and especially for the treatment of disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

The abnormalities and disorders which may be treated according to the present invention include any malignant, pre-malignant and non-malignant abnormalities or disorders responsive to photochemotherapy eg. tumours or other growths, skin disorders such as psoriasis or actinic keratoses, skin abrasions, and other diseases or infections eg. bacterial, viral or fungal infections, for example Herpes virus infections. The invention is particularly suited to the treatment of diseases, disorders or abnormalities where discrete lesions are formed to which the compositions may be directly applied (lesions is used here in a broad sense to include tumours and the like).

The internal and external body surfaces which may be treated according to the invention include the skin and all other epithelial and serosal surfaces, including for example mucosa, the linings of organs eg. the respiratory, gastro-intestinal and genito-urinary tracts, and glands with ducts which empty onto such surfaces (e.g. liver, hair follicles with sebaceous glands, mammary glands, salivary glands and seminal vesicles). In addition to the skin, such surfaces include for example the lining of the vagina, the endometrium and the urothelium. Such surfaces may also include cavities formed in the body following excision

- 10 -

of diseased or cancerous tissue eg. brain cavities following the excision of tumours such as gliomas.

Exemplary surfaces thus include: (i) skin and conjunctiva; (ii) the lining of the mouth, pharynx, oesophagus, stomach, intestines and intestinal appendages, rectum, and anal canal; (iii) the lining of the nasal passages, nasal sinuses, nasopharynx, trachea, bronchi, and bronchioles; (iv) the lining of the ureters, urinary bladder, and urethra; (v) the lining of the vagina, uterine cervix, and uterus; (vi) the parietal and visceral pleura; (vii) the lining of the peritoneal and pelvic cavities, and the surface of the organs contained within those cavities; (viii) the dura mater and meninges; (ix) any tumors in solid tissues that can be made accessible to photoactivating light e.g. either directly, at time of surgery, or via an optical fibre inserted through a needle.

The compositions of the invention may be formulated in conventional manner with one or more physiologically acceptable carriers or excipients, according to techniques well known in the art. Compositions may be administered topically, orally or systemically. Topical compositions are preferred, and include gels, creams, ointments, sprays, lotions, salves, sticks, soaps, powders, pessaries, aerosols, drops and any of the other conventional pharmaceutical forms in the art.

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will, in general, also contain one or more emulsifying, dispersing, suspending, thickening or colouring agents. Powders may be formed with the aid of any suitable powder base. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing, solubilising or suspending agents. Aerosol sprays are conveniently delivered from

- 11 -

pressurised packs, with the use of a suitable propellant.

Alternatively, the compositions may be provided in a form adapted for oral or parenteral administration, for example by intradermal, subcutaneous, intraperitoneal or intravenous injection. Alternative pharmaceutical forms thus include plain or coated tablets, capsules, suspensions and solutions containing the active component optionally together with one or more inert conventional carriers and/or diluents, e.g. with corn starch, lactose, sucrose, microcrystalline cellulose, magnesium stearate, polyvinylpyrrolidone, citric acid, tartaric acid, water, water/ethanol, water/glycerol, water/sorbitol, water/polyethyleneglycol, propyleneglycol, stearylalcohol, carboxymethylcellulose or fatty substances such as hard fat or suitable mixtures thereof.

The concentration of the compounds of the invention in the compositions, depends upon the nature of the compound, the composition, mode of administration and the patient and may be varied or adjusted according to choice. Generally however, concentration ranges of 1 to 50% (w/w) are suitable. For therapeutic applications concentration ranges of 10 to 50% have been found to be suitable, eg. 15 to 30% (w/w).

Following administration to the surface, the area treated is exposed to light to achieve the photo-chemotherapeutic effect. The length of time following administration, at which the light exposure takes place will depend on the nature of the composition and the form of administration. This can generally be in the order of 0.5 to 48 hours, e.g. 1 to 10 hours.

The irradiation will in general be applied at a dose level of 40 to 200 Joules/cm², for example at 100 Joules/cm².

The wavelength of light used for irradiation may be selected to achieve a more efficaceous photochemo-

- 12 -

therapeutic effect. Conventionally, when porphyrins are used in photochemotherapy they are irradiated with light at about the absorption maximum of the porphyrin. Thus, for example in the case of the prior art use of ALA in photochemotherapy of skin cancer, wavelengths in the region 350-640 nm, preferably 610-635 nm were employed. However, by selecting a broad range of wavelengths for irradiation, extending beyond the absorption maximum of the porphyrin, the photosensitizing effect may be enhanced. Whilst not wishing to be bound by theory, this is thought to be due to the fact that when Pp, and other porphyrins, are exposed to light having wavelengths within its absorption spectrum, it is degraded into various photo-products including in particular photoporphyrin (PPp). PPp is a chlorin and has a considerable photo-sensitizing effect; its absorption spectrum stretches out to longer wavelengths beyond the wavelengths at which Pp absorbs ie. up to almost 700 nm (Pp absorbs almost no light above 650 nm). Thus in conventional photochemotherapy, the wavelengths used do not excite PPp and hence do not obtain the benefit of its additional photosensitizing effect. Irradiation with wavelengths of light in the range 500-700 nm has been found to be particularly effective. It is particularly important to include the wavelengths 630 and 690 nm.

A further aspect of the invention thus provides a method of photochemotherapeutic treatment of disorders or abnormalities of external or internal surfaces of the body, comprising administering to the affected surfaces, a composition as hereinbefore defined, and exposing said surfaces to light, preferably to light in the wavelength region 300-800 nm, for example 500-700 nm.

Methods for irradiation of different areas of the body, eg. by lamps or lasers are well known in the art (see for example Van den Bergh, Chemistry in Britain, May 1986 p. 430-439).

- 13 -

The compounds of the invention may be formulated and/or administered with other photosensitizing agents, for example ALA or photofrin, or with other active components which may enhance the photochemotherapy effect. For example, chelating agents may beneficially be included in order to enhance accumulation of Pp; the chelation of iron by the chelating agents prevents its incorporation into Pp to form haem by the action of the enzyme ferrochelatase, thereby leading to a build-up of Pp. The photosensitizing effect is thus enhanced.

Aminopolycarboxylic acid chelating agents are particularly suitable for use in this regard, including any of the chelants described in the literature for metal detoxification or for the chelation of paramagnetic metal ions in magnetic resonance imaging contrast agents. Particular mention may be made of EDTA, CDTA (cyclohexane diamine tetraacetic acid), DTPA and DOTA. EDTA is preferred. To achieve the iron-chelating effect, desferrioxamine and other siderophores may also be used, e.g. in conjunction with aminopolycarboxylic acid chelating agents such as EDTA.

The chelating agent may conveniently be used at a concentration of 1 to 20% eg. 2 to 10% (w/w).

Additionally, it has been found that surface-penetration assisting agents and especially dialkylsulphoxides such as dimethylsulphoxide (DMSO) may have a beneficial effect in enhancing the photochemotherapy effect. This is described in detail in our co-pending application No. PCT/GB94/01951, a copy of the specification of which is appended hereto.

The surface-penetration assisting agent may be any of the skin-penetration assisting agents described in the pharmaceutical literature e.g. HPE -101 (available from Hisamitsu), DMSO and other dialkylsulphoxides, in particular n-decylmethyl-sulphoxide (NDMS), dimethylsulphacetamide, dimethylformamide (DMFA), dimethylacetamide, glycols, various pyrrolidone

- 14 -

derivatives (Woodford et al., J. Toxicol. Cut. & Ocular Toxicology, 1986, 5: 167-177), and Azone® (Stoughton et al., Drug Dpv. Ind. Pharm. 1983, 9: 725-744), or mixtures thereof.

DMSO however has a number of beneficial effects and is strongly preferred. Thus, in addition to the surface-penetration assisting effect (DMSO is particularly effective in enhancing the depth of penetration of the active agent into the tissue), DMSO has anti-histamine and anti-inflammatory activities. In addition, DMSO has been found to increase the activity of the enzymes ALA-synthase and ALA-dehydrogenase (the enzymes which, respectively, form and condense ALA to porphobilinogen) thereby enhancing the formation of the active form, Pp.

The surface penetration agent may conveniently be provided in a concentration range of 2 to 50% (w/w), eg about 10% (w/w).

According to the condition being treated, and the nature of the composition, the compounds of the invention may be co-administered with such other optional agents, for example in a single composition or they may be administered sequentially or separately. Indeed, in many cases a particularly beneficial photochemotherapeutic effect may be obtained by pre-treatment with the surface-penetration assisting agent in a separate step, prior to administration of the compounds of the invention. Furthermore, in some situations a pre-treatment with the surface-penetration assisting agent, followed by administration of the photochemotherapeutic agent in conjunction with the surface-penetration assisting agent may be beneficial. When a surface-penetration assisting agent is used in pre-treatment this may be used at high concentrations, e.g. up to 100% (w/w). If such a pre-treatment step is employed, the photochemotherapeutic agent may subsequently be administered up to several hours

- 15 -

following pre-treatment eg. at an interval of 5-60 minutes following pre-treatment.

Viewed from a further aspect, the invention thus provides a product comprising a compound of the invention or a pharmaceutically acceptable salt thereof, together with at least one surface-penetration assisting agent, and optionally one or more chelating agents as a combined preparation for simultaneous, separate or sequential use in treating disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

Alternatively viewed, this aspect of the invention also provides a kit for use in photochemotherapy of disorders or abnormalities of external or internal surfaces of the body comprising:

- a) a first container containing a compound of the invention or a pharmaceutically acceptable salt thereof,
- b) a second container containing at least one surface penetration assisting agent; and optionally
- c) one or more chelating agents contained either within said first container or in a third container.

Where the surface penetration agent is applied in a separate pre-treatment step, it may be applied in higher concentration, for example up to 100% (w/w).

It will be appreciated that the method of therapy using compounds of the invention inevitably involves the fluorescence of the disorder or abnormality to be treated. Whilst the intensity of this fluorescence may be used to eliminate abnormal cells, the localization of the fluorescence may be used to visualize the size, extent and situation of the abnormality or disorder. This is made possible through the surprising ability of

- 16 -

ALA esters to preferentially localize to non-normal tissue.

The abnormality or disorder thus identified or confirmed at the site of investigation may then be treated through alternative therapeutic techniques e.g. surgical or chemical treatment, or by the method of therapy of the invention by continued build up of fluorescence or through further application of compounds of the invention at the appropriate site. It will be appreciated that diagnostic techniques may require lower levels of fluorescence for visualization than used in therapeutic treatments. Thus, generally, concentration ranges of 1 to 50% e.g. 1-5% (w/w) are suitable. Sites, methods and modes of administration have been considered before with regard to the therapeutic uses and are applicable also to diagnostic uses described here. The compounds of the invention may also be used for in vitro diagnostic techniques, for example for examination of the cells contained in body fluids. The higher fluorescence associated with non-normal tissue may conveniently be indicative of an abnormality or disorder. This method is highly sensitive and may be used for early detection of abnormalities or disorders, for example bladder or lung carcinoma by examination of the epithelial cells in urine or sputum samples, respectively. Other useful body fluids which may be used for diagnosis in addition to urine and sputum include blood, semen, tears, spinal fluid etc. Tissue samples or preparations may also be evaluated, for example biopsy tissue or bone marrow samples. The present invention thus extends to the use of compounds of the invention, or salts thereof for diagnosis according to the aforementioned methods for photochemotherapy, and products and kits for performing said diagnosis.

A further aspect of the invention relates to a method of in vitro diagnosis, of abnormalities or

- 17 -

disorders by assaying a sample of body fluid or tissue of a patient, said method comprising at least the following steps:

- i) admixing said body fluid or tissue with a compound as described hereinbefore,
- ii) exposing said mixture to light,
- iii) ascertaining the level of fluorescence, and
- iv) comparing the level of fluorescence to control levels.

The invention will now be described in more detail in the following non-limiting Examples, with reference to the drawings in which:

Figure 1 shows fluorescence intensity (relative units vs wavelength (nm)) of PpIX in the normal skin of mice after topical administration of

- (A) free ALA
- (B) ALA methylester
- (C) ALA ethylester
- (D) ALA propylester

after 0.5, 1, 1.5, 2.5, 3, 3.5 and 14 hours following administration;

Figure 2 shows the distribution of PpIX as measured by fluorescence intensity (relative units vs wavelength (nm)) in Brain, dermis, Ear, Liver and muscle 14 hours after topical administration to the normal skin of mice:

- (A) free ALA
- (B) ALA methylester
- (C) ALA ethylester
- (D) ALA propylester;

Figure 3 shows PpIX fluorescence (fluorescence intesity, relative units vs wavelength (nm)) in the skin of mice

- 18 -

15 minutes, 1 hour, 4 hours and 10 hours after intraperitoneal injection of ALA methylester (150 mg/kg);

Figure 4 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) (A) 1.5 hours and (B) 4 hours after topical administration of ALA methylester to basal cell carcinoma (BCC) lesions on the skin of human patients (- tumour; --- normal skin);

Figure 5 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) (A) 1.5 hours and (B) 4 hours after topical administration of ALA ethylester to basal cell carcinoma (BCC) lesions on the skin of human patients (- tumour; --- normal skin);

Figure 6 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) (A) 1.5 hours and (B) 4 hours after topical administration of ALA propylester to basal cell carcinoma (BCC) lesions on the skin of human patients (- tumour; --- normal skin);

Figure 7 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) (A) 1.5 hours and (B) 4 hours after topical administration of ALA to basal cell carcinoma (BCC) lesions on the skin of human patients (- tumour; --- normal skin);

Figure 8 shows measurement of PpIX production following topical application of ALA methylester in human BCC and surrounding normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

Figure 9 shows measurement of PpIX production following topical application of ALA in human BCC and surrounding

- 19 -

normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

Figure 10 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) 24 hours following topical administration of ALA methylester to BCC lesion and to normal skin of human patients.

Figure 11 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) 24 hours following topical administration of ALA to BCC lesion and to normal skin of human patients.

Figure 12 shows measurement of PpIX production 4.5 hours following topical application of ALA methylester in human BCC by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

Figure 13 shows measurement of PpIX production 4.5 hours following topical application of ALA methylester in human normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

Figure 14 shows measurement of PpIX production 24 hours following topical application of ALA methylester in human BCC by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

Figure 15 shows measurement of PpIX production 24 hours following topical application of ALA methylester in human normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

- 20 -

Figure 16 shows measurement of PpIX production 24 hours following topical application of free ALA in human BCC by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

Figure 17 shows measurement of PpIX production 24 hours following topical application of free ALA in human normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

Figure 18 shows measurement of PpIX production 4.5 hours following topical application of free ALA and 20% DMSO in human BCC by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

Figure 19 shows measurement of PpIX production 4.5 hours following topical application of free ALA and 20% DMSO in human normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

Figure 20 shows a time course (fluorescence intensity relative units vs time (hours)) of ALA methylester-induced (PpIX) fluorescence in the mouse skin after topical application of ALA methylester alone (-•-), ALA methylester plus DMSO (-▲-), ALA methylester plus desferrioxamine (DF) (-■-) or ALA methylester plus DF and DMSO (-▼-). Each point is the mean of measurements from at least three mice;

Figure 21 shows fluorescence photographs of the mouse skin taken 1 h after topical application of free ALA alone (A), ALA methylester (B), ALA ethylester (C) and ALA propylester (D), showing fluorescence in the

- 21 -

epidermis (Ep), epithelial hair follicles and sebaceous gland (arrows), but not in the dermis (De). Original magnification x250.

Figure 22 is a graph showing relative tumour volume against time (days) following treatment of WiDr human colonic carcinoma transplanted subcutaneously into nude mice with ALA or ALA methylester plus DF; (-▲-) control; (-▼-) DF alone; (-■-) ALA + DF + DMSO; (-●-) ALA methylester + DF + DMSO.

Figure 23 shows PpIX fluorescence ratios between BCC lesions and surrounding normal skin after topical application of ALA or its esters.

Example 1

Preparation of methyl 5-aminolevulinate hydrochloride

To a 500 ml glass reactor containing 200 ml methanol, was added 1g 5-amino-levulinic acid hydrochloride and 1 drop conc. HCl. The reaction mixture was then stirred overnight at 60°C. The progress of the esterification was followed by ¹H-NMR. Excess methanol was removed by distillation, and the product further dried under vacuum at 30-40°C, giving methyl 5-aminolevulinate hydrochloride. The structure was confirmed by ¹H-NMR in DMSO-d₆.

Example 2

Preparation of ethyl 5-aminolevulinate hydrochloride
(ALA ethylester)

1g 5-aminolevulinic acid hydrochloride was added to 200 ml dry ethanol containing 1-2 drops conc. hydrochloric acid in a 250 ml glass reactor equipped with a stirrer, reflux condenser and a thermometer. The esterification was performed at reflux overnight (70-80°C). After the

- 22 -

reaction had gone to completion, the ethanol was removed under vacuum. Finally, the product was dried under high vacuum at 30-40°C, giving 0.94g Ethyl 5-aminolevulinate hydrochloride. Confirmation of the structure was done by $^1\text{H-NMR}$ in DMSO-d₆.

Example 3

Preparation of n-propyl 5-aminolevulinate hydrochloride
(ALA propylester)

0.5g 5-aminolevulinic acid hydrochloride was dissolved in 100 ml dry n-propanol containing 1-2 drops of conc. hydrochloride in a 250 ml glass reactor equipped with a stirrer, reflux condenser and a thermometer. The reaction mixture was stirred at 70-80°C for approx. 20 hours. After all the 5-aminolevulinic acid was converted to its n-propylester (followed by $^1\text{H-NMR}$), the excess propanol was removed, and the product dried under high vacuum (<1 mBar) at 40-50°C. The reaction gave 0.49g propyl 5-aminolevulinate hydrochloride. The structure was confirmed by $^1\text{H-NMR}$ in DMSO-d₆.

Example 4

Preparation of n-hexyl 5-aminolevulinic hydrochloride
(ALA hexylester)

2 grams of 5-aminolevulinic acid hydrochloride was dissolved in 25 grams of dry n-hexanol with 5-6 drops of conc. hydrochloride added in a 50 ml glass reactor equipped with a reflux condenser and a thermometer. The reaction mixture was held at 50-60°C for approx. 3 days. The excess n-hexanol was removed under vacuum and the product finally dried under high vacuum, giving 2.4 grams of n-hexyl 5-aminolevulinate hydrochloride. The structure was confirmed by $^1\text{H-NMR}$ spectroscopy in DMSO-d₆.

- 23 -

Example 5

Preparation of n-heptyl 5-aminolevulinic hydrochloride
(ALA heptylester)

0.5g 5-aminolevulinic acid hydrochloride was added to 30 grams of n-heptanol containing 5 drops of conc. hydrochloride in a 100 ml glass reactor equipped with a stirrer, reflux condenser and a thermometer. After all the 5-aminolevulinic acid had dissolved, the reaction mixture was stirred at 70-80°C for approx. 48 hours. After the 5-aminolevulinic acid was converted to its n-heptyl ester (followed by $^1\text{H-NMR}$), the excess alcohol was removed, and the product dried under high vacuum (< 1 mBar) at 70°C. The reaction gave 1.5g n-heptyl 5-aminolevulinate hydrochloride. The structure was confirmed by $^1\text{H-NMR}$ in DMSO-d_6 .

Example 6

Preparation of n-octyl 5-aminolevulinic hydrochloride
(ALA octylester)

1 gram 5-aminolevulinic acid hydrochloride was added to 30 grams of dry n-octanol containing 5-6 drops of conc. hydrochloride in a 50 ml glass reactor equipped with a reflux condenser, stirrer and a thermometer. The reaction mixture was stirred at 65-70°C for approx. 2 days. Excess n-octanol was removed under vacuum and the product finally dried under high vacuum, giving 1.5 grams of n-octyl 5-aminolevulinate hydrochloride. The structure was confirmed by $^1\text{H-NMR}$ spectroscopy in DMSO-d_6 .

Example 7

Formulation

20% creams were prepared by admixture of the active component, ALA, ALA methylester, ALA ethylester, or ALA

- 24 -

propylester (prepared according to Examples 1 to 3 respectively), with "Urguentum Merck" cream base (available from Merck) consisting of silicon dioxide, paraffin liq., vaseline, album, cetostearol., polysorbat. 40, glycerol monostearate, Miglyol®812 (a mixture of plant fatty acids), polypropyleneglycol., and purified water.

Corresponding creams were also prepared, additionally containing 3-20% DMSO.

Example 8

Determination of protoporphyrin IX production in the skin of mice by CCD microscopy of biopsies:

A commercial oil-in-water cream containing (20% w/w) one of the chemicals (free ALA, ALA methylester, ALA ethylester and ALA propylester) (see Example 1) was topically applied to the normal skin of nu/nu nude mice for 0.5, 1, 3 and 6 hours, then biopsied and evaluated by means of microscopic fluorescence photometry incorporating a light-sensitive thermol-electrically cooled charge coupled device (CCD) camera. The results show that free ALA and its three ester derivatives are taken up by the skin tissue, the esterified ALA derivatives are being deesterified in the skin, and converted into protoporphyrin IX (PpIX) 0.5 hours after topical application. The fluorescence intensity of PpIX in the skin increased with the time of the application and the maximum amounts of the fluorescence were seen about 6 hours (the latest time point studied) after the application in all cases.

Example 9

Measurements in situ of protoporphyrin IX production in the skin of mice by an optical-fiber based system

- 25 -

The aim of this study was to investigate the build-up of esterified ALA ester-induced porphyrins fluorescence in the normal skin of nude mice *in vivo* after topical or systemic administration of ALA ester derivatives.

MATERIALS AND METHODS

Chemicals. 5-aminolevulinic acid (ALA) methyl-, ethyl- and propyl-esters ($H_2N-CH_2COOCH_2-CH_2COO-R$; R can be CH_3 , $CH_2-CH_2-CH_3$) were prepared by Norsk Hydro Research Center (Porsgrunn, Norway) as described in Examples 1 to 3.

Free ALA hydrochloride and desferrioxamine mesylate (DF) were purchased from Sigma Chemical Company (St. Louis, Mo, USA). Dimethyl sulphoxide (DMSO) was obtained from Janssen Chimica (Geel, Belgium). Commercial oil-water creams (Unguentum Merck, Darmstadt, Germany) containing 20% one of the ALA ester derivatives (w/w), 20% free ALA, 20% ALA methylester plus 5% DF, 20% ALA methylester plus 20% DMSO, or 20% ALA methylester plus 5% DF and 20% DMSO were freshly prepared prior to use. All creams were made by the Pharmacy at the Norwegian radium Hospital. For intraperitoneal injection, ALA and its methylester were freshly dissolved in saline. All other chemicals used were of the highest purity commercially available.

Animals. Female Balb/c nu/nu athymic nude mice were obtained from the Animal Laboratory at the Norwegian Radium Hospital and kept under specific-pathogen-free conditions. At the start of the experiments the mice were 6-7 weeks old weighing 18-24 g. Three mice were housed per cage with autoclaved covers in a dark room during the experiments.

Treatment procedure. One of the creams was painted on the normal skin at right flank region of each mouse, and covered by a semi-permeable dressing (3M, St Paul, MN,

- 26 -

USA) for various time intervals (from 0.25 to 24 h) before fluorescence measurements *in situ* or being biopsied for microscopic fluorescence imaging. About 0.2g cream was applied to an approximate 2.25 cm² area of the skin. In the case of i.p. injection the mice were given ALA or its methylester at a dose of 150 mg/kg. At least three mice were used for each condition.

Fluorescence spectroscopic measurements in situ. A perkin Elmer LS-50 fluorescence spectrometer equipped with a red-sensitive photomultiplier (Hamamatsu R 928) was used. This instrument has a pulsed Xenon arc light source and phase sensitive detection, such that fluorescence can be readily measured. Part of the excitation beam (set at 408 nm for fluorescence measurements) was reflected into a 600 µm core multimodus optical quartz fiber (No. 3501 393, Dornier Medizintechnik, GmbH, Germerring, Germany) by means of a mirror for application onto the subject through a hand held probe. Emission in the region of 550-750 nm was measured via emission fibres collecting information through the probe.

Fluorescence microscopy. After the creams were topically applied to the skin of mice for various times (as indicated above), the skin was biopsied and the frozen tissue sections were cut with a cryostat to a thickness of 8 µm. The fluorescence microscopy was carried out using an Axioplan microscope (Zeiss, Germany) with a 100 W mercury lamp. The fluorescence images were recorded by a light-sensitive thermo-electrically cooled charge coupled device (CCD) camera (resolution: 385x578 pixels with a dynamic range of 16 bits per pixel) (Astromed CCD 3200, Cambridge, UK) and hard copies on a video printer (Sony multiscan video printer UP-930). The filter combination used for detection of porphyrin fluorescence consisted of 390-440

- 27 -

nm excitation filter, a 460 nm beam splitter and a >600 nm emission filter.

Results

PpIX fluorescence was measured *in situ* by an optical-fiber based system in the normal skin of nude mice 0.5, 1, 1.5, 2.5, 3, 3.5 and 14 hours after topical application of free ALA or one of its ester derivatives as described above. As shown in Figure 1, the PpIX fluorescence was already built-up 1 hour after topical application in the case of all derivatives, while the fluorescence was seen 1.5 hours after the application of free ALA. The maximum fluorescence intensity was found 14 hours after the application in all cases, but PpIX fluorescence induced from ALA esters in the skin was stronger than that from free ALA. Furthermore, as can be seen in Figure 2, 14 hours after the application no fluorescence of ALA-esters-induced PpIX was detected in other areas of the skin and internal organs including ear, dermis, muscle, brain and liver. However, in the case of free ALA, a strong fluorescence was also seen in the ear as well as in the other areas of the skin. Thus, after topical application ALA-ester-induced PpIX was found locally in the skin, whereas free ALA-induced PpIX distributed not only locally, but also in other areas of the skin. We suggest that ALA is transported in the blood and that PpIX is subsequently formed in all organs containing the enzymes of the heme synthesis pathway and/or PpIX is formed in the skin and then transported to other tissues via blood circulation. The latter situation may lead to skin photosensitivity in areas where free ALA is not topically applied. In addition, after intraperitoneal injection of ALA methylester at a dose of 150 mg/kg, the PpIX fluorescence in the skin of mice was built-up 15 minutes after the injection and the peak value was found around

4 hours, and the fluorescence disappeared within 10 hours post the injection (Figure 3). This kinetic pattern is similar to that of the fluorescence of free ALA-induced porphyrins in the skin following i.p. injection of the same dose, although the fluorescence decreased faster in the case of the ester than in the case of the free ALA.

Example 10

Measurements of protoporphyrin IX production in human basal cell carcinoma (BCC) and surrounding normal skin by optical-fiber based system

The PpIX fluorescence in the BCC lesions and surrounding normal skin of human patients was measured *in situ* by optical-fiber based system after topical application of 20% free ALA and its derivatives for various time intervals.

Figures 4, 5, 6 and 7 show that, compared to free ALA, the ALA derivatives-induced PpIX was built up faster, produced more and localized more selectively in the BCC lesions (i.e. much less fluorescence in the surrounding normal skin), particularly for ALA methylester.

Example 11

In vivo fluorescence surface measurements of PpIX production in human BCC and surrounding normal skin by CCD microscopy of biopsies

In a 78 years old Caucasian male presenting multiple ulcero-nodular BCCs lesions were exposed to commercial oil-in-water creams containing either ALA alone (20% w/w) or ALA methyl ester (20% w/w) (as described in Example 7) covered by a semi-permeable dressing for 24 hours. After removal of dressings and cream *in vivo* fluorescence was measured at the surface of tumor tissue

and adjacent normal skin by means of a spectrofluorometer. Punch biopsies of the same areas were removed and samples were immediately immersed in liquid nitrogen. The tissue sections were cut with a cryostat microtome to a thickness of 8 μm . The localization pattern of the porphyrin fluorescence in the tissue sections was directly observed by means of fluorescence microscopy. The same frozen sections were subsequently stained with routine H&E staining for histological identification. The same sections were subsequently stained with routine H&E staining for histological identification. Fluorescence microscopy was carried out with an Axioplan microscope (Zeiss, Germany). Fluorescence images and quantitative measurements were performed by a light-sensitive thermol-electrically cooled charge coupled device (CCD) camera (Astromed CCD 3200, Cambridge, UK) and an image processing unit (Astromed/Visilog, PC 486DX2 66 MHz VL). The main purpose for such quantitative measurements is to determine the exact penetration of ALA-induced porphyrins from tissue surface to the bottom layers of cancer lesions. The results are shown in Figures 8 and 9 in which the fluorescence intensity is expressed as a function of depth of cancer lesion.

As shown in Figures 8 and 9, an homogeneous distribution of PpIX fluorescence is seen from the top to the bottom of the whole BCC lesions after use of either free ALA or its methyl ester. This suggests that ALA methylester is at least as good as free ALA in terms of penetration and PpIX production in the BCC lesion. In addition, no PpIX fluorescence was seen in the surrounding normal skin after topical application of ALA methylester, indicating that ALA-methylester-induced PpIX highly selectively took place only in the BCC lesion.

In vivo fluorescence after 24 hours showed at least

- 30 -

doubled fluorescence intensity for ALA methyl ester compared to ALA for the selected tumors and also an increase for corresponding normal tissues, however this only of about 50%. The ratio between tumor and normal tissue was about 1.2: 1 for ALA and 2: 1 for the ALA methyl ester. The results are shown in Figures 10 and 11.

At control one week after treatment all treatment fields presented a central necrotic area corresponding to the tumor. In the adjacent normal skin exposed to cream and light irradiation there was observed a marked erythema for the ALA while for the ALA methyl ester only moderate erythema was observed.

Example 12

In vivo fluorescence surface measurements of PpIX production in human BCC and surrounding normal skin by CCD microscopy of biopsies

The present data show the localization patterns and production of porphyrins (mainly protoporphyrin IX (PpIX)) after topical application of free ALA and one of its derivatives (methyl ester) for 4.5 and 24 hours in the nodular basal cell carcinomas (BCCs) and surrounding normal skin of patients. The tests were performed as described in Example 11.

Each of the following figures show both (A) fluorescence images of either the bottom layer of BCC lesions or of the surrounding normal skin. Curves indicating the fluorescence intensity as a function of depth of the BCC lesions or of the normal skin are also shown (B).

Figure 12 shows a homogenous distribution of PpIX fluorescence generated by ALA methyl ester in the bottom

- 31 -

layer of a BCC 4.5 hours after topical application. There is also some porphyrin fluorescence in surrounding normal skin (Figure 13). The fluorescence intensity ratio between BCC and the normal skin is about 2. Moreover, the absolute amount of the fluorescence induced by ALA methyl ester is higher than that induced by free ALA and 20% DMSO after topical application for 4.5 hours, as shown below.

Figures 14 and 15 show a uniform distribution of porphyrin fluorescence induced by topical application of ALA methyl ester for 24 hours in the bottom layer of BCC and surrounding normal skin. The ratio of the fluorescence in BCC and that in normal skin is also about 2. Furthermore, the fluorescence intensity of ALA methyl ester-induced porphyrins in the BCC is almost twice as high as that in BCC after topical application of free ALA alone for 24 hours, as shown below.

Figures 16 and 17 show a homogenous distribution of free ALA-induced porphyrins in the bottom layer of BCC and surrounding normal skin 24 following topical application. However, the ratio of the fluorescence intensity between BCC and normal skin is about 1, which indicates a low selectivity of this treatment. Moreover the production of porphyrins in BCC is less than that in the case of ALA methyl ester.

Figures 18 and 19 show a homogenous distribution of ALA-induced porphyrins in the bottom layer of BCC and surrounding normal skin after topical application of free ALA and 20% DMSO for 4.5 hours. However, the ratio of the fluorescence intensity between BCC and normal skin is only slightly larger than 1, which demonstrates that the DMSO probably reduces the tumor selectivity of the porphyrins produced. Moreover, also in this case less porphyrins are produced in BCC than in the case of

- 32 -

the application of ALA methyl ester.

Example 13

Investigation of the effects of the chelating agent desferrioxamine (DF) and/or DMSO and fluorescence of skin

I. The effect of DF and/or DMSO on the build up of fluorescence in the normal skin of mice in situ was ascertained various times after topical administration of ALA-methylester. Methods were performed as described in Example 9.

RESULTS

Topical application of the cream alone containing only DMSO did not show any fluorescence in the normal mouse skin, but there was some fluorescence of PpIX after DF alone was applied.

DF or DF plus DMSO (a well-known skin penetration enhancer) significantly enhanced the production of ALA methylester-induced PpIX.

II. Fluorescence imaging of the skin treated with three derivatives (performed as described in Example 9) showed fluorescence of the ester derivative-induced porphyrins in the epidermis, epithelial hair follicles and sebaceous glands 1 h after topical application (Figure 21). The fluorescence intensity of the porphyrins increased with the time after the application.

SUMMARY

A large number of patients with basal cell carcinomas (BCCs) has topically been treated with ALA-based PDT in our hospital during the past five years and more than 90% of superficial BCCs have shown a complete

- 33 -

regression. However, nodular BCCs had a low complete response rate due to a poor ALA retention and, consequently, a low ALA-induced porphyrin production in the deep layers of the lesions. In order to improve the technique, we used ALA ester derivatives instead of free ALA. The present data obtained presented in this Example and in Example 9 by means of both fluorescence spectroscopic measurements in situ and fluorescence microscopy of tissue biopsies, indicate that all three ester derivatives studied were taken up, de-esterified and finally converted into porphyrins in the epidermis, epithelial hair follicles and sebaceous glands of the nude mice with a higher porphyrin production than that of free ALA. This is in agreement with the preceding Examples concerning a study of human nodular basal cell carcinoma that demonstrate that the fluorescence of the ALA ester-induced porphyrins was built up faster with a higher intensity and a more homogenous distribution than those of free ALA-induced porphyrins in the lesions.

The present study also shows that DF has a significant effect in enhancing the production of ALA methylester-derived PpIX in the normal skin of the mice after topical application.

Interestingly, a strong fluorescence of free ALA-induced porphyrins was found in regions of the skin outside the area where the cream was topically applied (Figure 2). This indicates that after topical application free ALA is transported in the blood and porphyrins are subsequently formed in all organs containing the enzymes of the heme synthesis pathway or porphyrins are initially formed in the skin or/and liver, then transported to other tissues via blood circulation. This may lead to skin photosensitivity in areas where free ALA is even not topically applied. However, none of the ester derivatives studied induced porphyrin

- 34 -

fluorescence in other parts of the skin.

Example 14

Effects of ALA methylester or ALA, DF and DMSO PDT on tumor growth in WiDr human colonic carcinoma-transplanted nude mice

Nude mice were transplanted with WiDr human colonic carcinoma cells by subcutaneous injection into the right flank region. The following creams, formulated as described in the preceding Examples, were applied topically to the site of the tumor: 10% DF alone; 20% ALA + 10% DF + 20% DMSO; or 20% ALA methylester + 10% DF + 20% DMSO, followed, 14 hours later by laser light irradiation (632 nm, 150 mW/cm² for 15 minutes). A separate group of animals bearing the same tumor model, but receiving no topical application of the cream, served as a control. The responses of the treated tumors were evaluated as tumor regression/regrowth time. When the tumors reached a volume 5 times that of the volume on the day of light irradiation, the mice were killed. The results are shown in Figure 22. (Bars: standard error of mean (SEM) based on 3-5 individual animals in each group). The results show that it took 34 days for tumors treated with ALA methylester + DF + DMSO to reach a volume five times that of the volume on the day just before light irradiation, whereas in the case of free ALA + DF + DMSO it took 24 days for the treated tumors to grow to 5 times size. Thus, ALA methylester is more effective than ALA in slowing tumor regrowth.

EXAMPLE 15

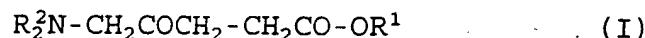
Selectivity of ALA esters (methyl, hexyl, heptyl and octyl) for non-normal tissue

- 35 -

The PpIX fluorescence ratios between BCC lesions and surrounding normal skin after topical application of ALA or its esters (20% for 4 hours), was examined using methods described in previous examples. The results are shown in Fig.23 and indicate that all esters can more selectively induce PpIX in BCC lesions than free ALA, particularly in the case of ALA-methylester and ALA-hexylester.

Claims:

1. Compounds being esters of 5-aminolevulinic acids or pharmaceutically acceptable salts thereof.
2. Compounds of formula I,



(wherein R¹ may represent alkyl optionally substituted by hydroxy, alkoxy, acyloxy, alkoxy carbonyloxy, amino, aryl, oxo or fluoro groups and optionally interrupted by oxygen, nitrogen, sulphur or phosphorus atoms; and R², each of which may be the same or different, represents a hydrogen atom or a group R¹) and salts thereof.

3. Compounds as claimed in claim 2 wherein the aryl group is phenyl or a monocyclic 5-7 membered heteroaromatic.
4. Compounds as claimed in claim 2 or 3 wherein R¹ represents an unsubstituted alkyl group and/or each R² represents a hydrogen atom.
5. Compounds as claimed in any one of claims 2 to 4 wherein the alkyl group contains up to 10 carbon atoms.
6. Compounds as claimed in any one of claims 2 to 5 wherein the compounds are ALA-methylester, ALA-ethylester, ALA-propyl ester, ALA-hexylester, ALA-heptylester or ALA-octylester or salts thereof.
7. A process for preparing the compounds as defined in any one of claims 1 to 6, comprising forming an ester of the carboxy group of a 5-aminolevulinic acid.
8. A process as claimed in claim 7, comprising

- 37 -

reacting a 5-aminolevulinic acid, or an esterifiable derivative thereof, with an alkanol or an ester-forming derivative thereof.

9. A process as claimed in claim 7 or 8, which process comprises at least one of the following steps:

(a) reacting a compound of formula II



(wherein X represents a leaving group, or COX represents an acid anhydride group and R² is as defined in claim 2)
with a compound of formula III



(wherein R¹ is as defined in claim 2); and

(b) converting a compound of formula I into a pharmaceutically acceptable salt thereof.

10. Compounds as claimed in any one of claims 1 to 9, or salts thereof, for use in photochemotherapy or diagnosis.

11. A pharmaceutical composition comprising a compound as defined in any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutical carrier or excipient.

12. The use of a compound as defined in any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, for the preparation of a therapeutic agent for use in photochemotherapy, or a diagnostic agent for use in diagnosis.

13. The use as claimed in claim 12 wherein the photochemotherapy or diagnosis is performed on disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

14. A method of diagnosis or photochemotherapeutic treatment of disorders or abnormalities of external or internal surfaces of the body, comprising administering to the sites of investigation or affected surfaces, a composition as defined in claim 11, and exposing said sites or surfaces to light.

15. A method as claimed in claim 14 wherein the light is in the wavelength region 500-700 nm.

16. A product comprising a compound as claimed in any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof, together with at least one surface-penetration assisting agent, and optionally one or more chelating agents as a combined preparation for simultaneous, separate or sequential use in treating or diagnosing disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

17. A product as claimed in claim 16 wherein the surface-penetration assisting agent is DMSO.

18. A method of in vitro diagnosis of abnormalities or disorders by assaying a sample of body fluid or tissue of a patient, said method comprising at least the following steps:

- i) admixing said body fluid or tissue with a compound as defined in any one of claims 1 to 9,
- ii) exposing said mixture to light,
- iii) ascertaining the level of fluorescence, and

- 39 -

iv) comparing the level of fluorescence to control levels.

19. A kit for use in diagnosis or photochemotherapy of disorders or abnormalities of external or internal surfaces of the body comprising:

- a) a first container containing a compound as claimed in any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof,
- b) a second container containing at least one surface penetration assisting agent; and optionally
- c) one or more chelating agents contained either within said first container or in a third container.

- 36 -

Claims:

1. Compounds being esters of 5-aminolevulinic acids or pharmaceutically acceptable salts thereof.
2. Compounds of formula I,



(wherein R¹ may represent alkyl optionally substituted by hydroxy, alkoxy, acyloxy, alkoxy carbonyloxy, amino, aryl, oxo or fluoro groups and optionally interrupted by oxygen, nitrogen, sulphur or phosphorus atoms; and R², each of which may be the same or different, represents a hydrogen atom or a group R¹) and salts thereof.

3. Compounds as claimed in claim 2 wherein the aryl group is phenyl or a monocyclic 5-7 membered heteroaromatic.
4. Compounds as claimed in claim 2 or 3 wherein R¹ represents an unsubstituted alkyl group and/or each R² represents a hydrogen atom.
5. Compounds as claimed in any one of claims 2 to 4 wherein the alkyl group contains up to 10 carbon atoms.
6. Compounds as claimed in any one of claims 2 to 5 wherein the compounds are ALA-methylester, ALA-ethylester, ALA-propyl ester, ALA-hexylester, ALA-heptylester or ALA-octylester or salts thereof.
7. A process for preparing the compounds as defined in any one of claims 1 to 6, comprising forming an ester of the carboxy group of a 5-aminolevulinic acid.
8. A process as claimed in claim 7, comprising

- 37 -

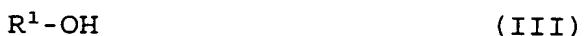
reacting a 5-aminolevulinic acid, or an esterifiable derivative thereof, with an alkanol or an ester-forming derivative thereof.

9. A process as claimed in claim 7 or 8, which process comprises at least one of the following steps:

(a) reacting a compound of formula II



(wherein X represents a leaving group, or COX represents an acid anhydride group and R² is as defined in claim 2)
with a compound of formula III



(wherein R¹ is as defined in claim 2); and

(b) converting a compound of formula I into a pharmaceutically acceptable salt thereof.

10. Compounds as claimed in any one of claims 1 to 9, or salts thereof, for use in photochemotherapy or diagnosis.

11. A pharmaceutical composition comprising a compound as defined in any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutical carrier or excipient.

12. The use of a compound as defined in any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, for the preparation of a therapeutic agent for use in photochemotherapy, or a diagnostic agent for use in diagnosis.

- 38 -

13. The use as claimed in claim 12 wherein the photochemotherapy or diagnosis is performed on disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

14. A method of diagnosis or photochemotherapeutic treatment of disorders or abnormalities of external or internal surfaces of the body, comprising administering to the sites of investigation or affected surfaces, a composition as defined in claim 11, and exposing said sites or surfaces to light.

15. A method as claimed in claim 14 wherein the light is in the wavelength region 500-700 nm.

16. A product comprising a compound as claimed in any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof, together with at least one surface-penetration assisting agent, and optionally one or more chelating agents as a combined preparation for simultaneous, separate or sequential use in treating or diagnosing disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

17. A product as claimed in claim 16 wherein the surface-penetration assisting agent is DMSO.

18. A method of in vitro diagnosis of abnormalities or disorders by assaying a sample of body fluid or tissue of a patient, said method comprising at least the following steps:

- i) admixing said body fluid or tissue with a compound as defined in any one of claims 1 to 9,
- ii) exposing said mixture to light,
- iii) ascertaining the level of fluorescence, and

- 39 -

- iv) comparing the level of fluorescence to control levels.

19. A kit for use in diagnosis or photochemotherapy of disorders or abnormalities of external or internal surfaces of the body comprising:

- a) a first container containing a compound as claimed in any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof,
- b) a second container containing at least one surface penetration assisting agent; and optionally
- c) one or more chelating agents contained either within said first container or in a third container.

1 / 23

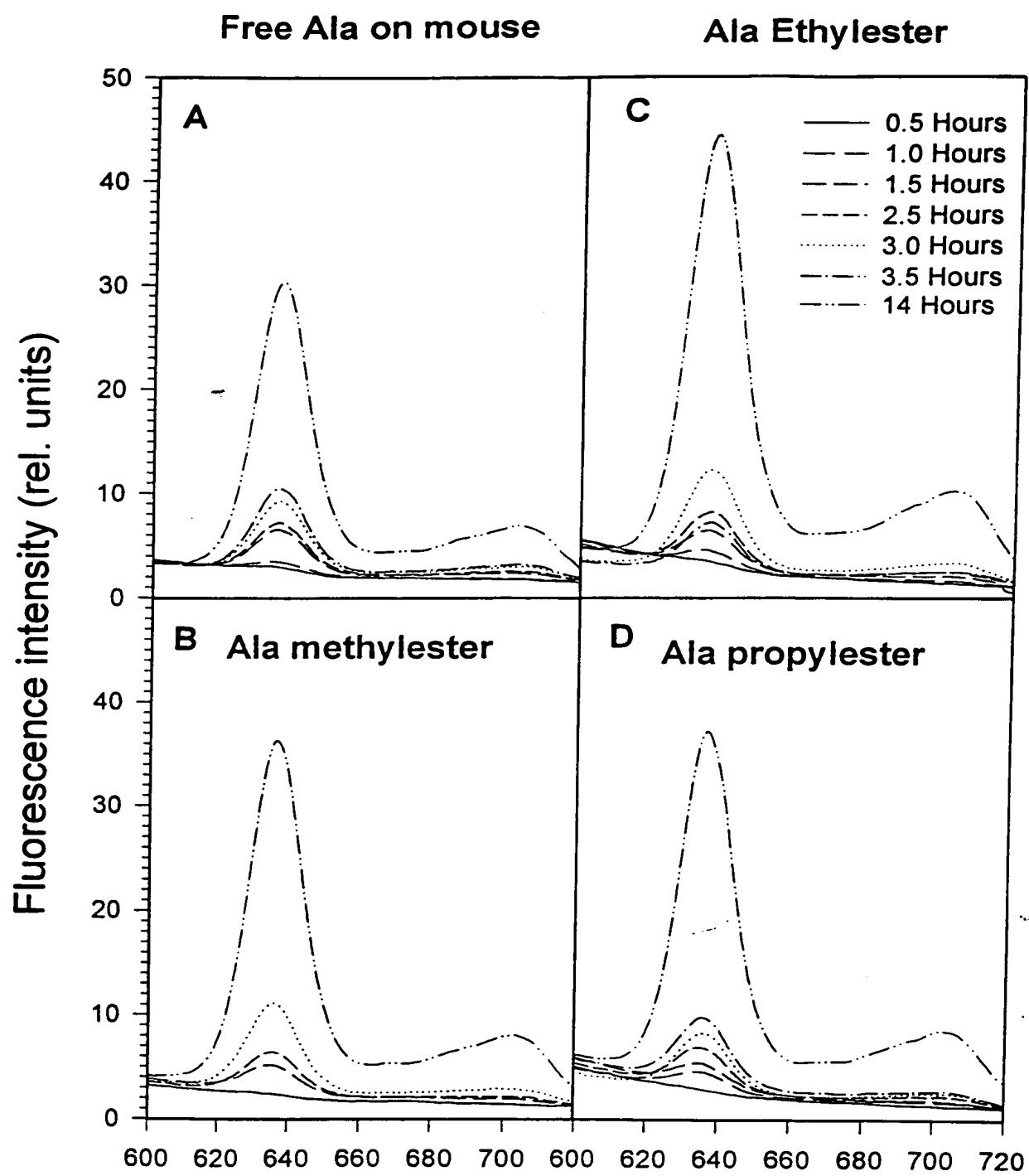


FIG. 1. Wavelength (nm)

2 / 23

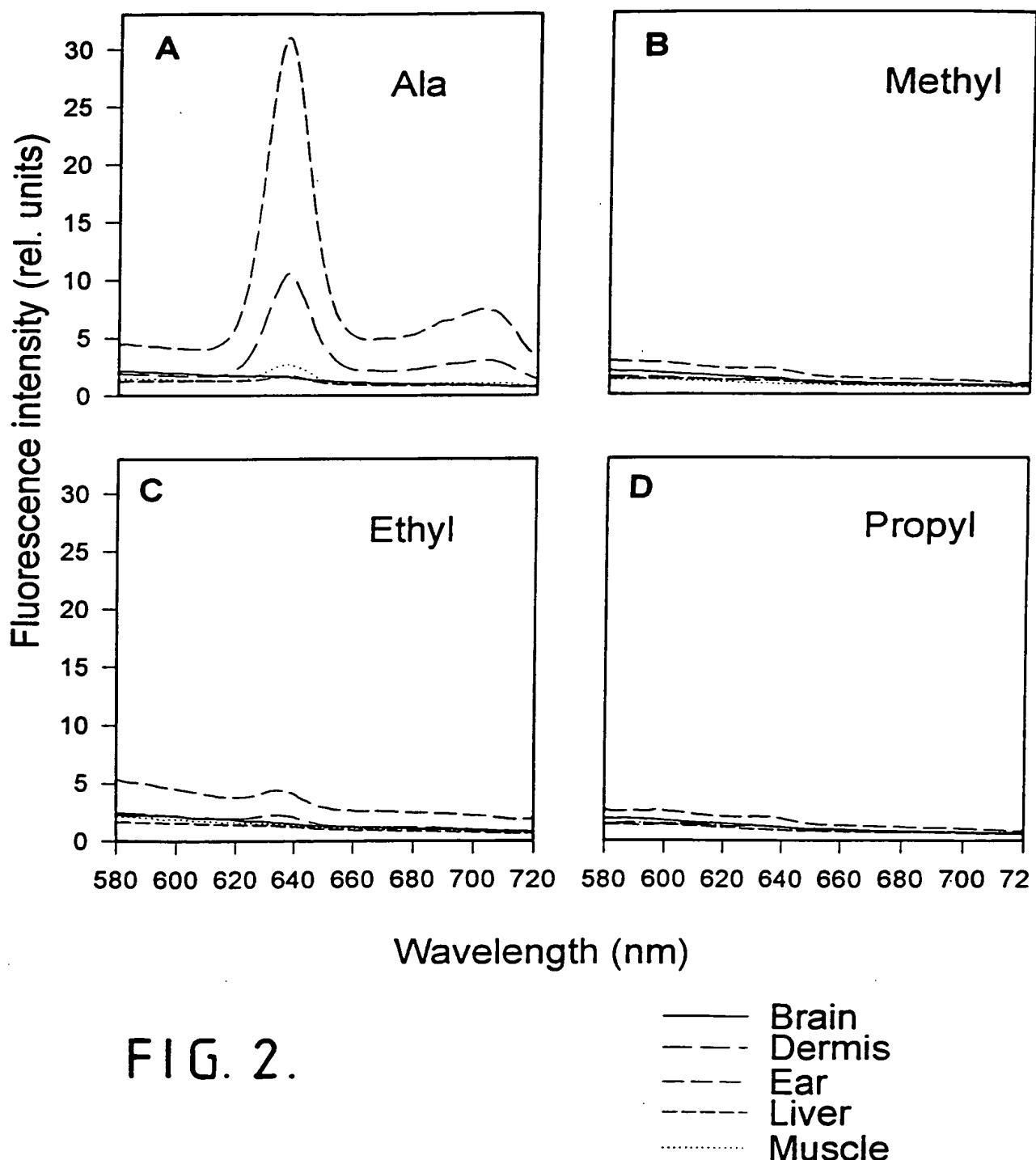


FIG. 2.

3 / 23

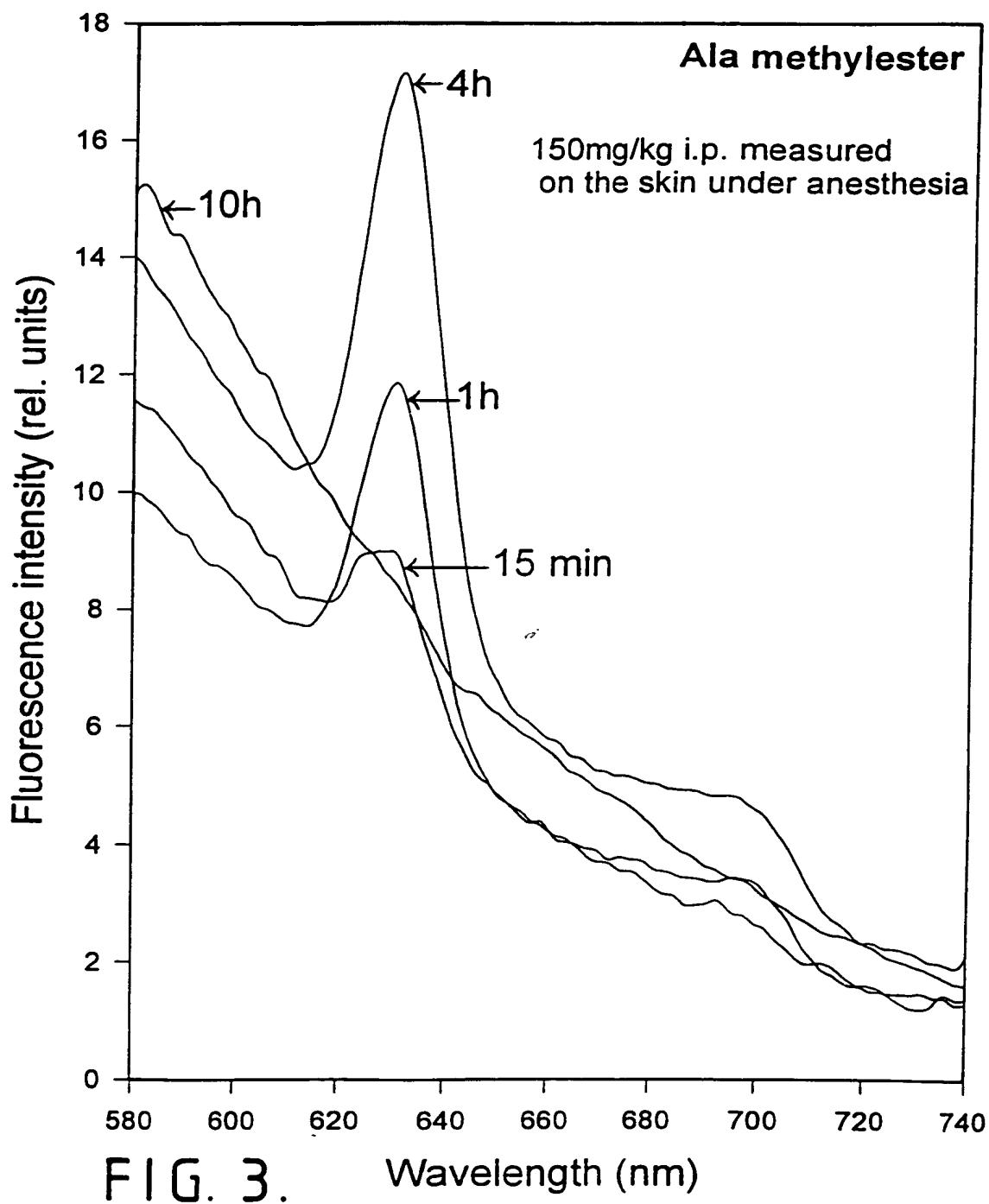


FIG. 3.

4 / 23

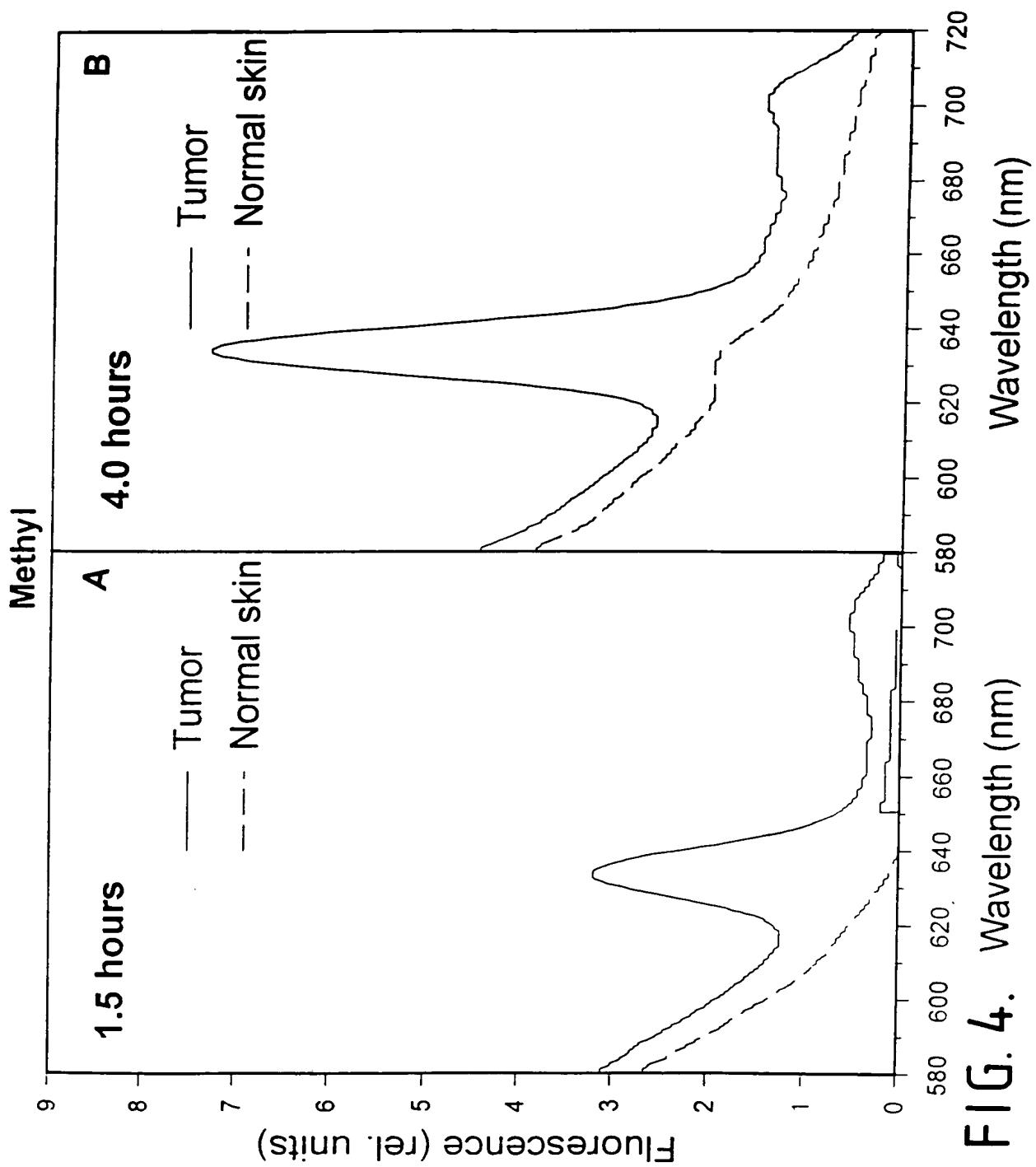


FIG. 4. Wavelength (nm)

5 / 23

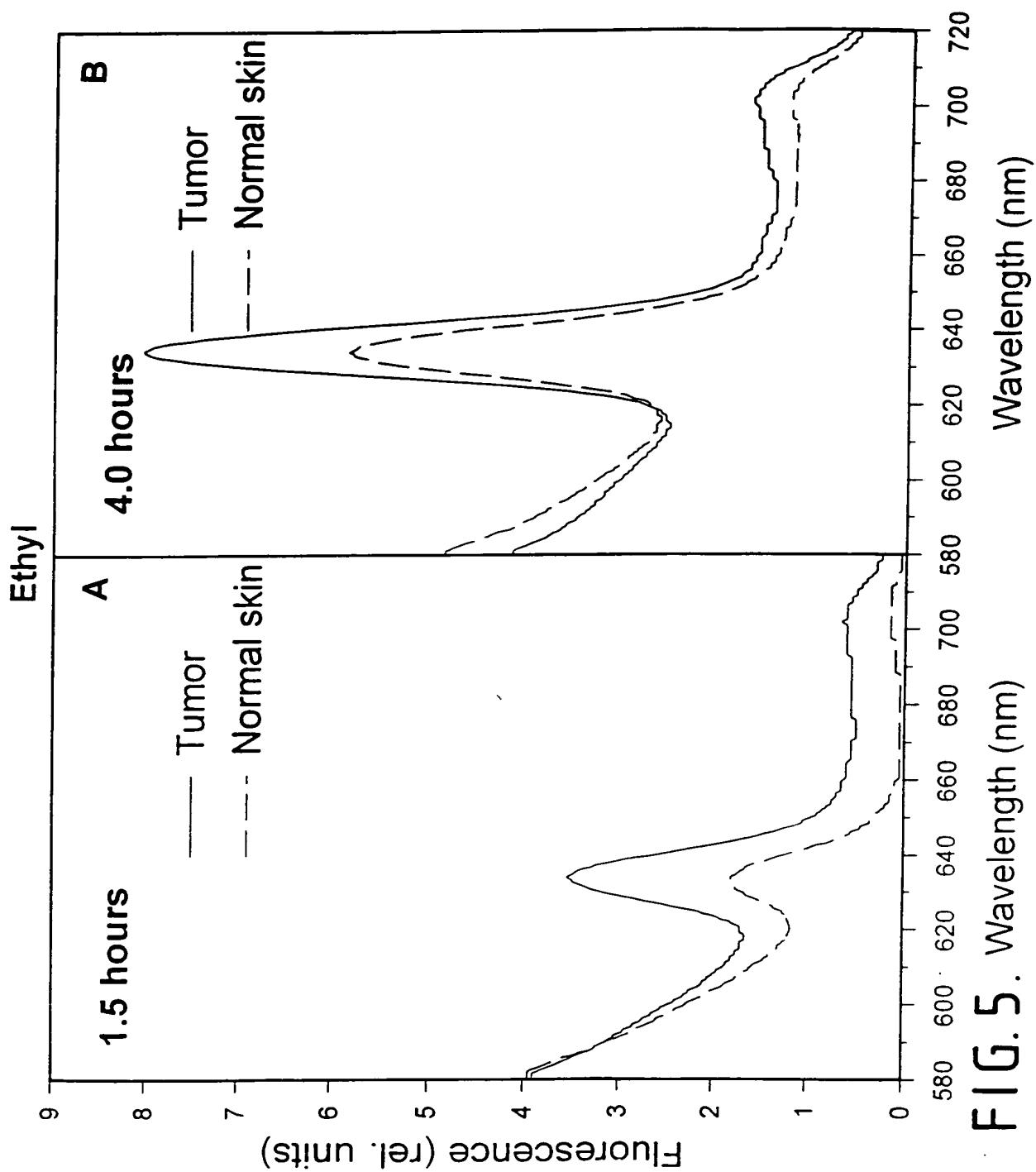


FIG. 5. Wavelength (nm)

6 / 23

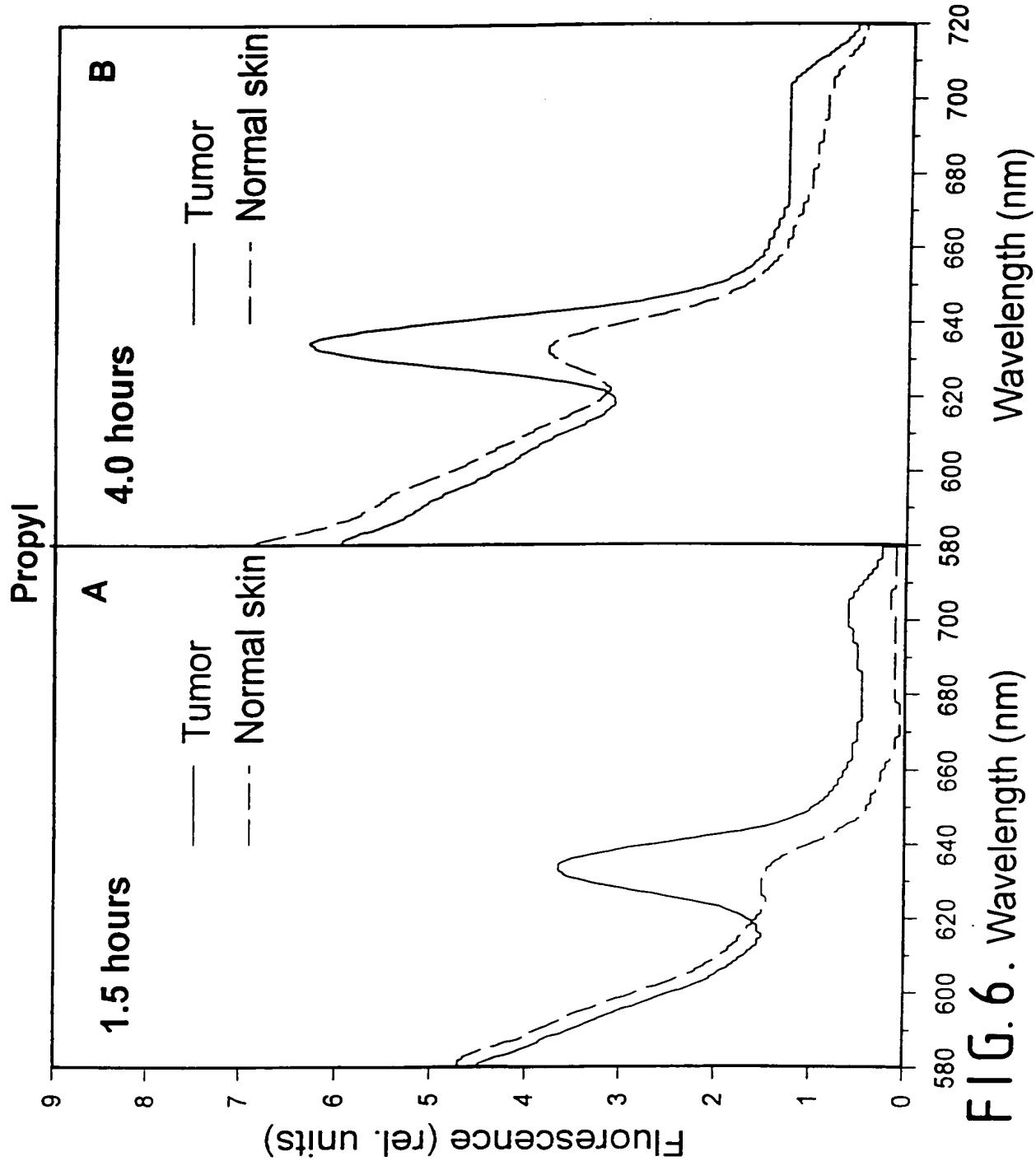


FIG. 6. Wavelength (nm)

7 / 23

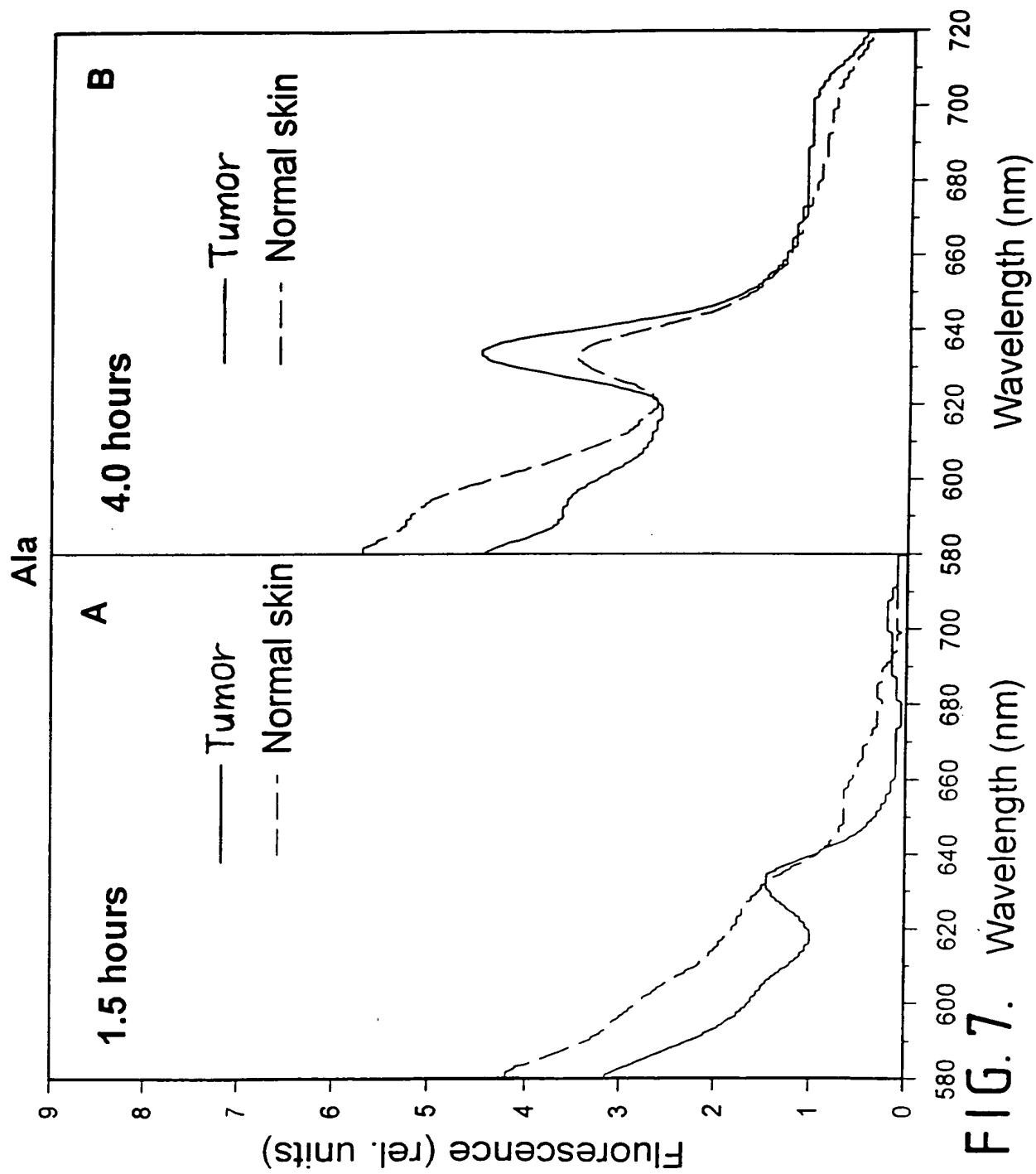
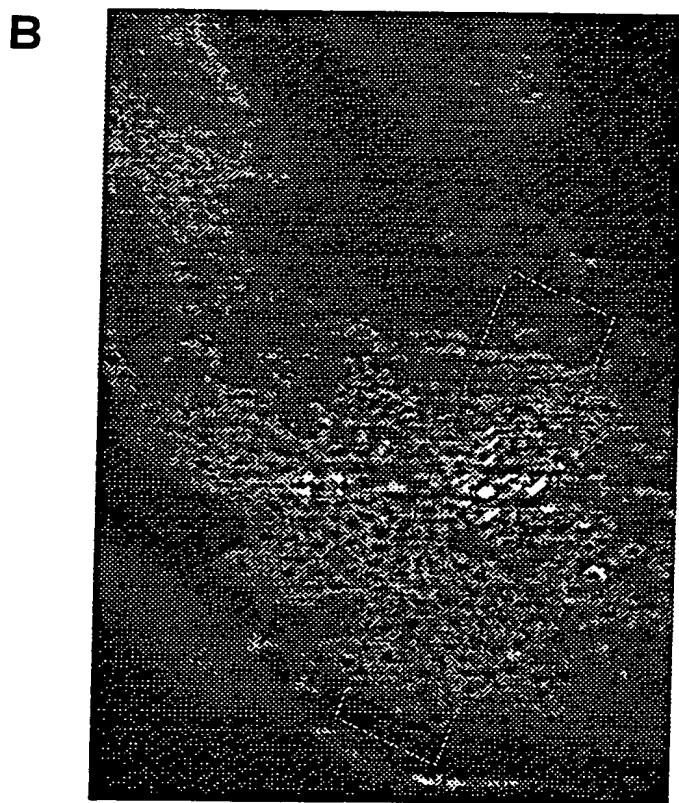
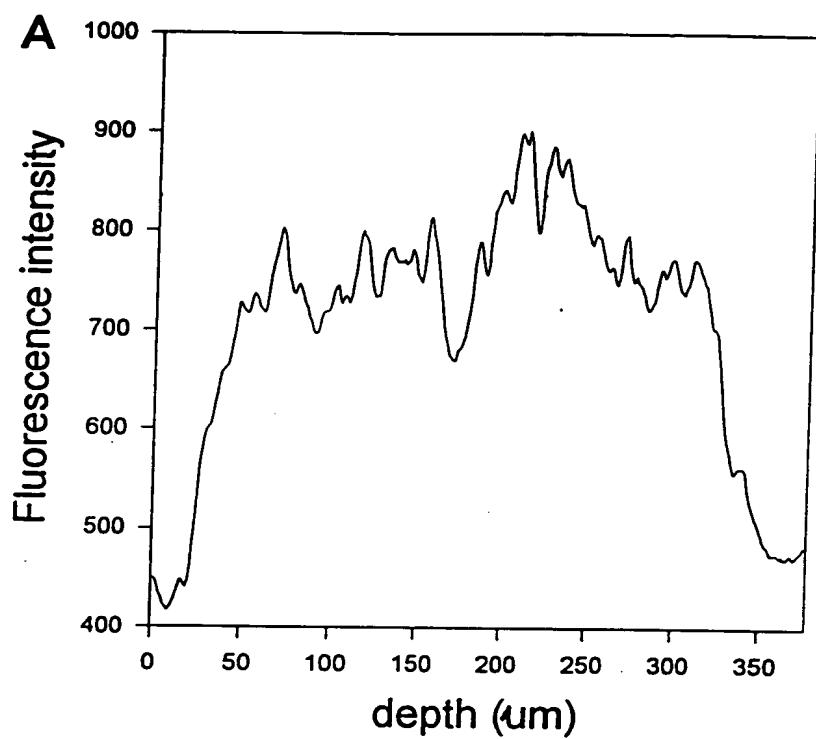


FIG. 7. Wavelength (nm)

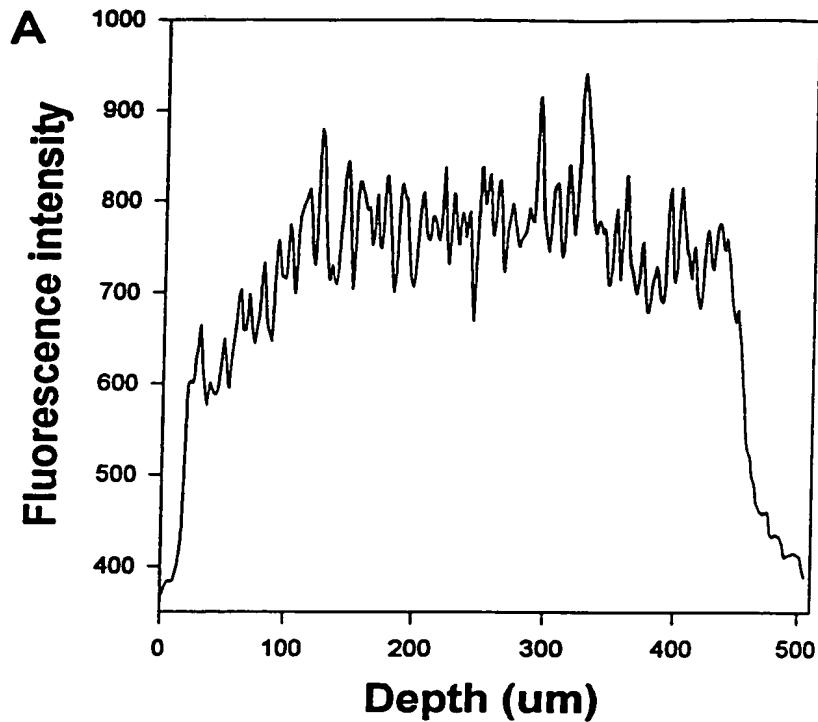
8 / 23



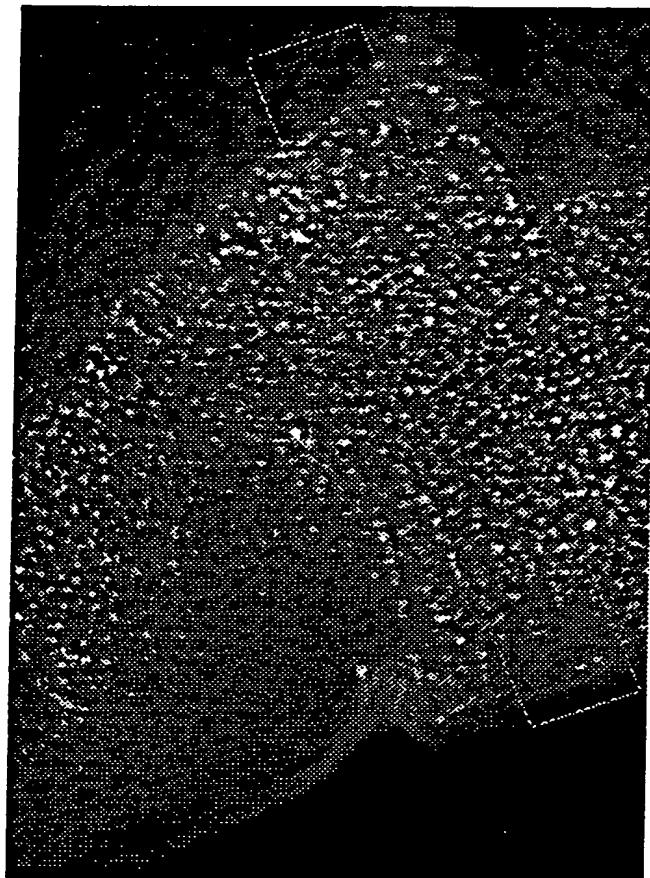
Free ALA

Figure 8

9 / 23

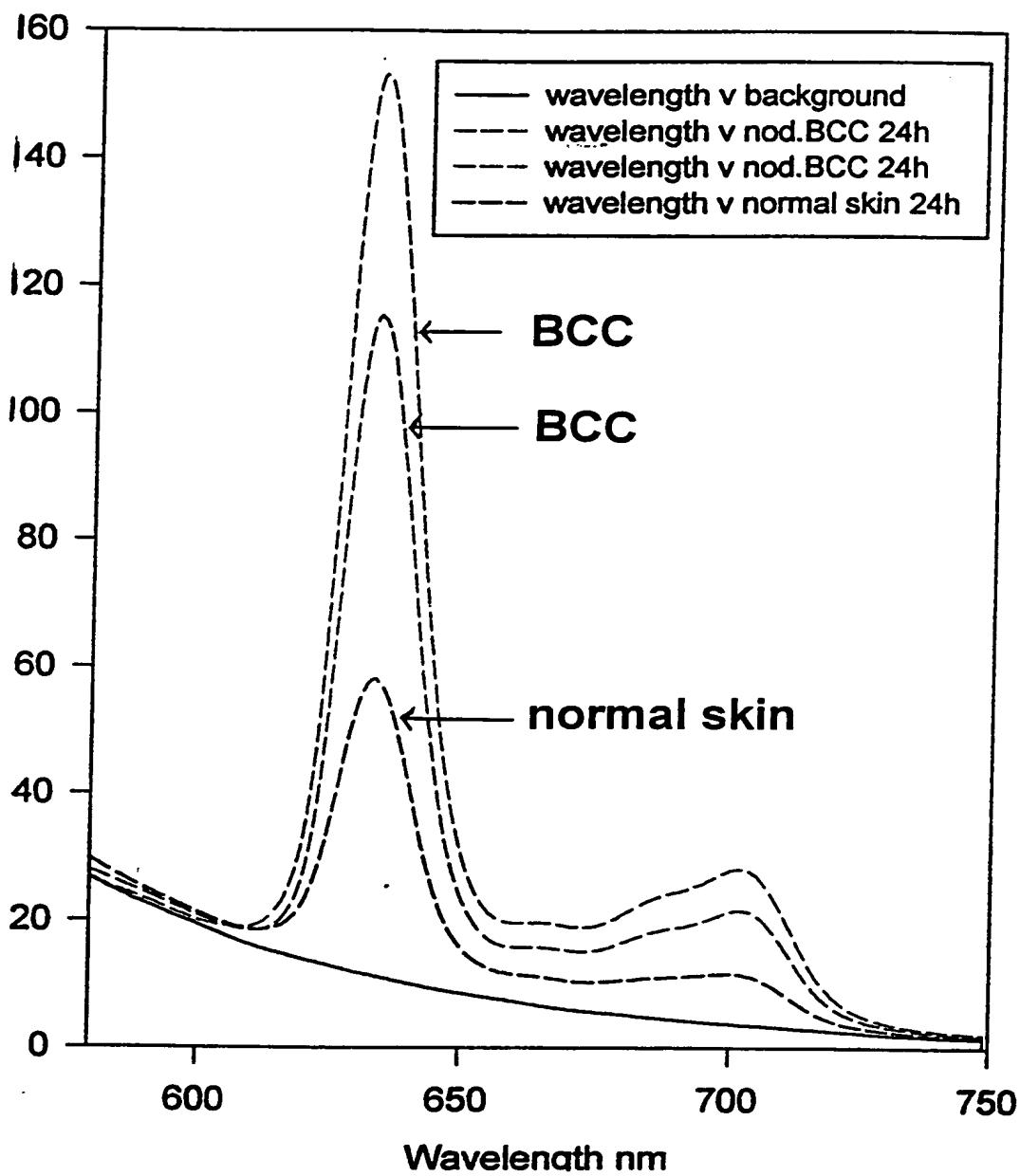


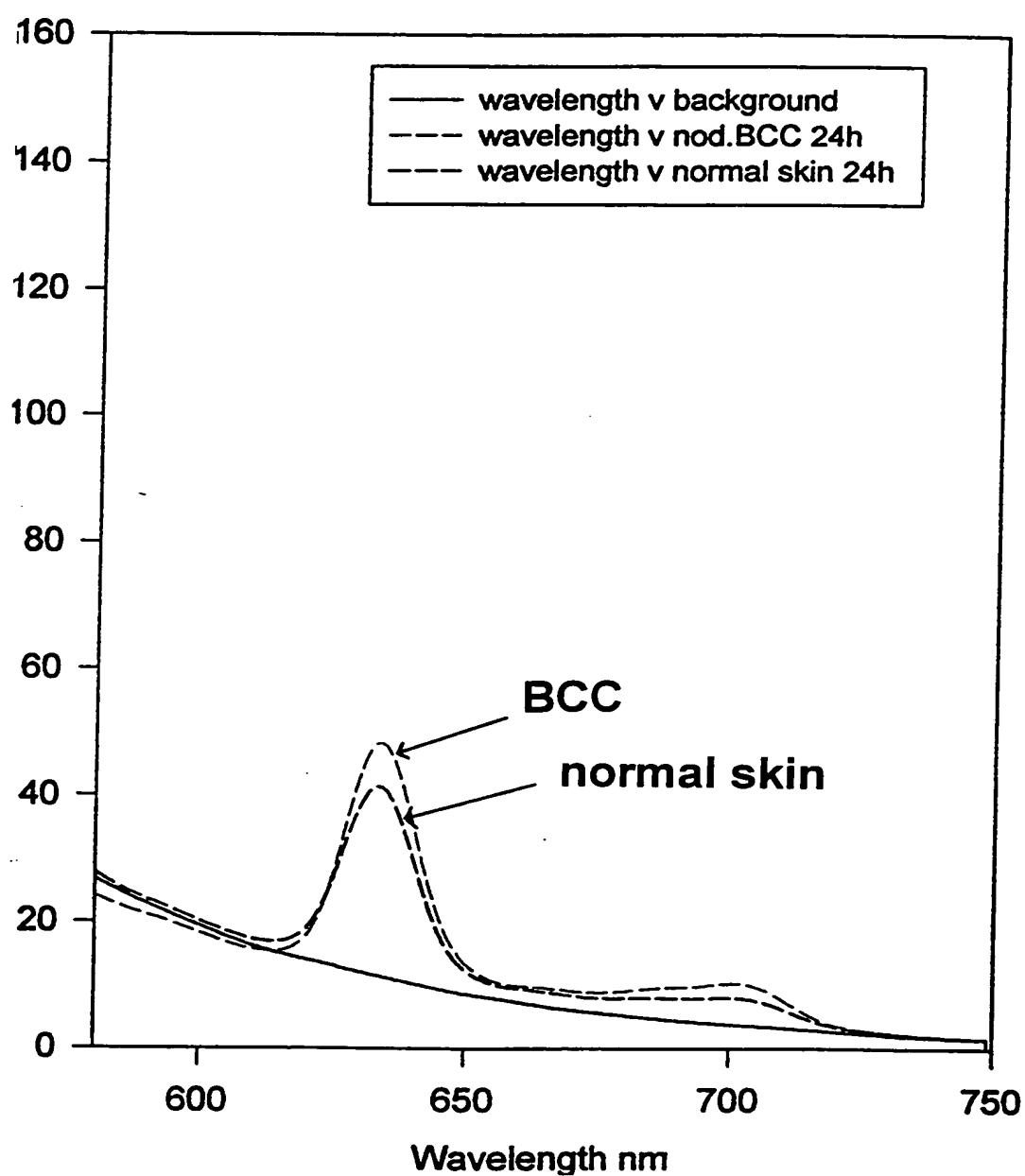
B



ALA methylester

Figure 9

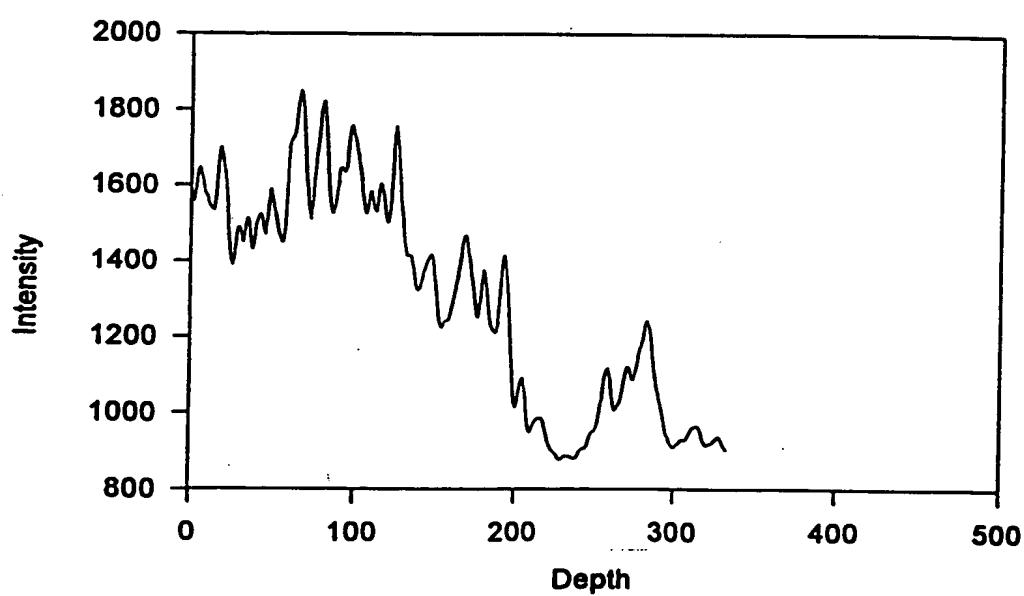
BCC ALA-methyl 24 hoursFigure 10

BCC ALA 24 hoursFigure 11

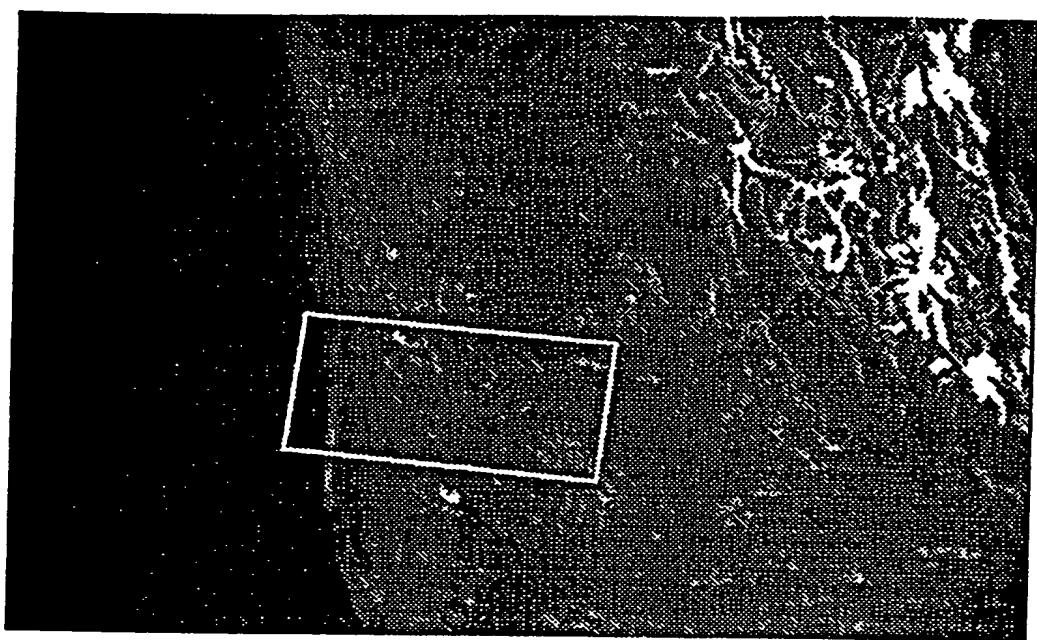
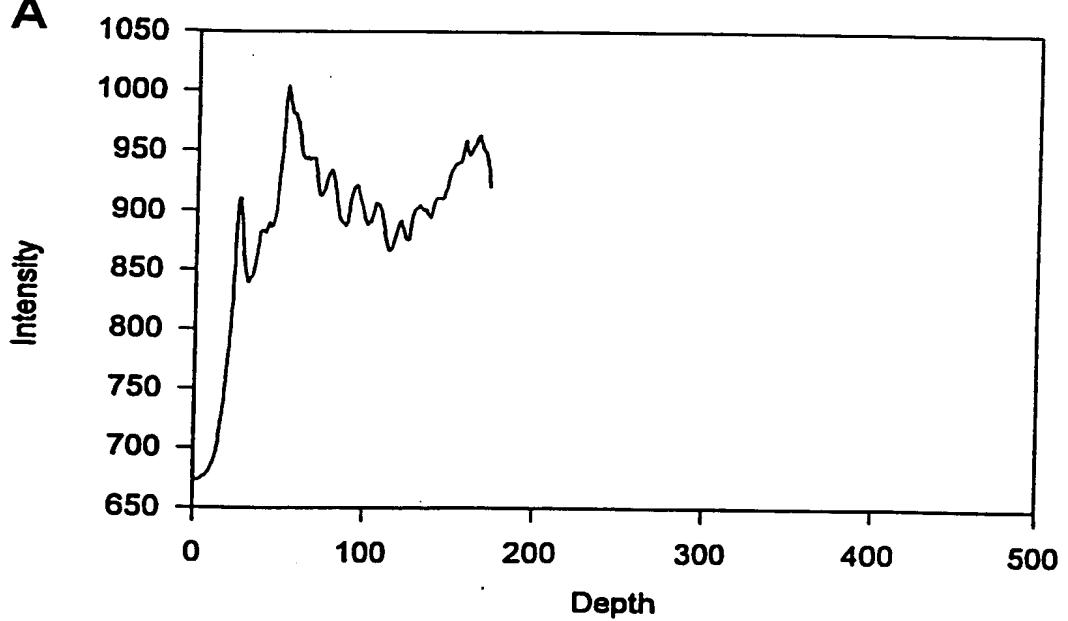
12 / 23

B**A**

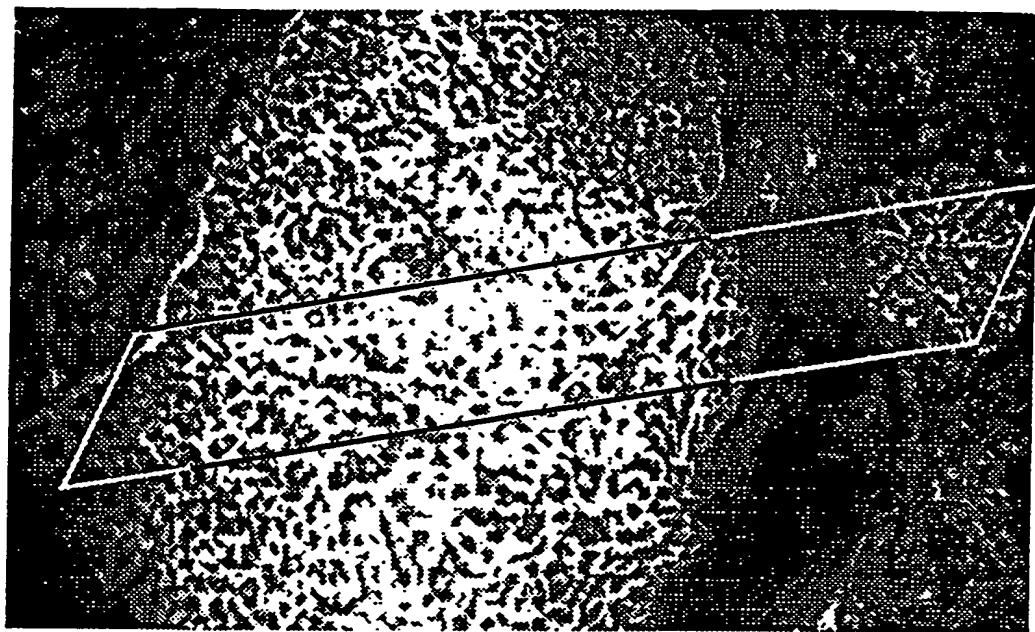
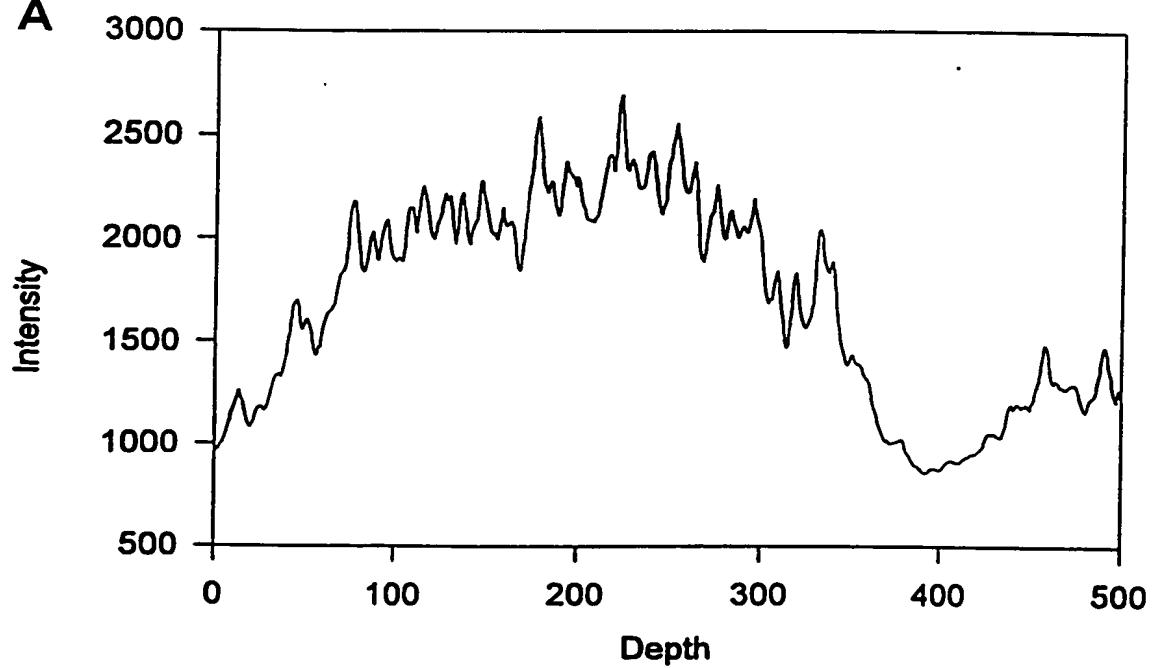
155-20

Figure 12

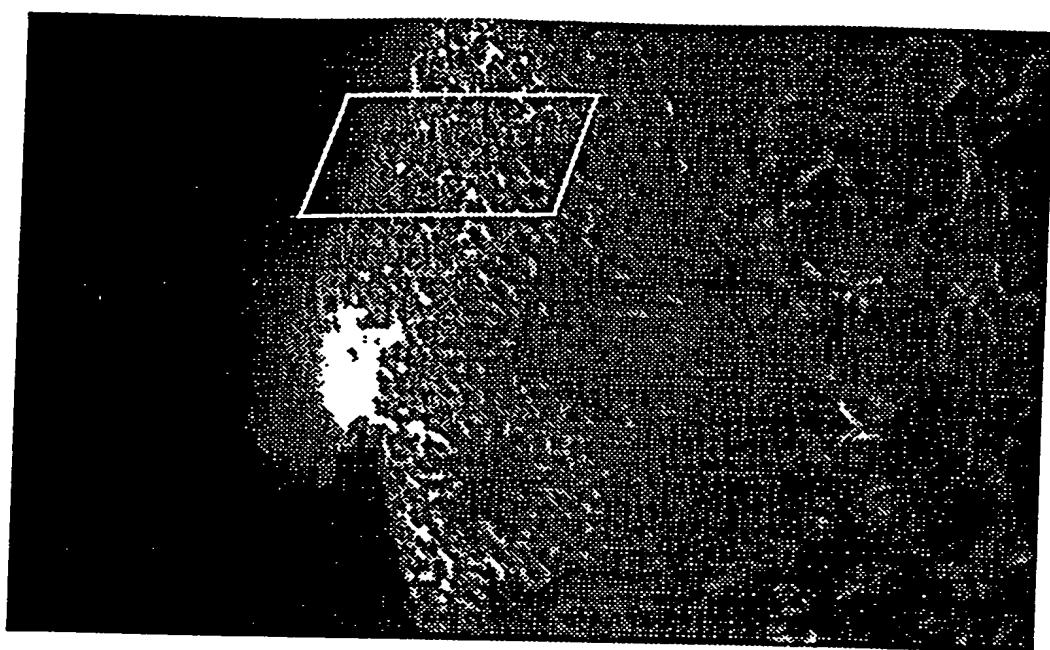
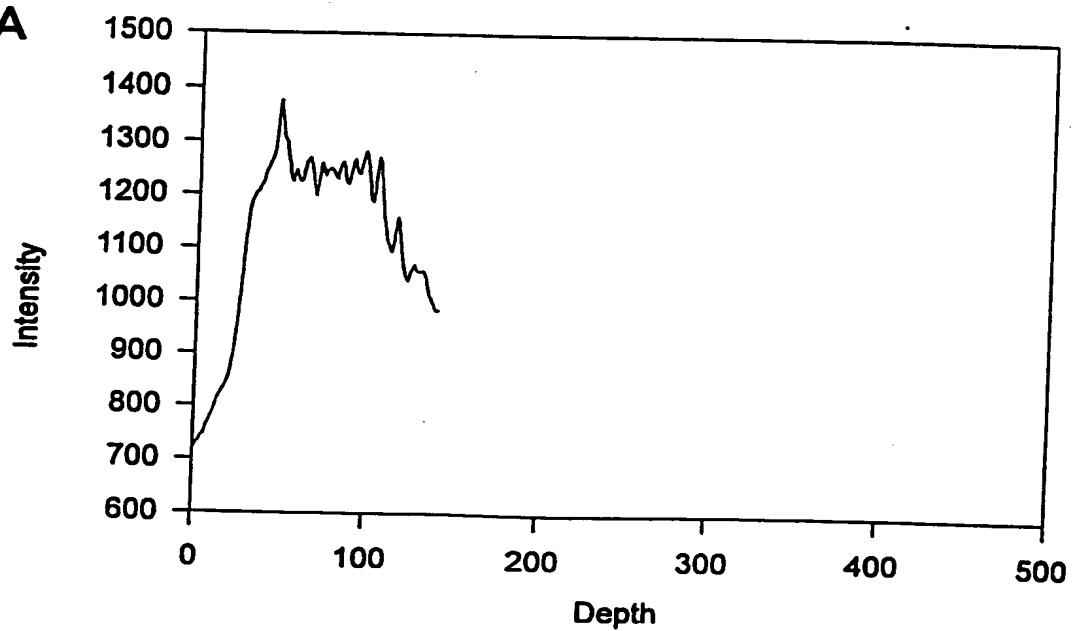
13 / 23

B**156-1****A****Figure 13**

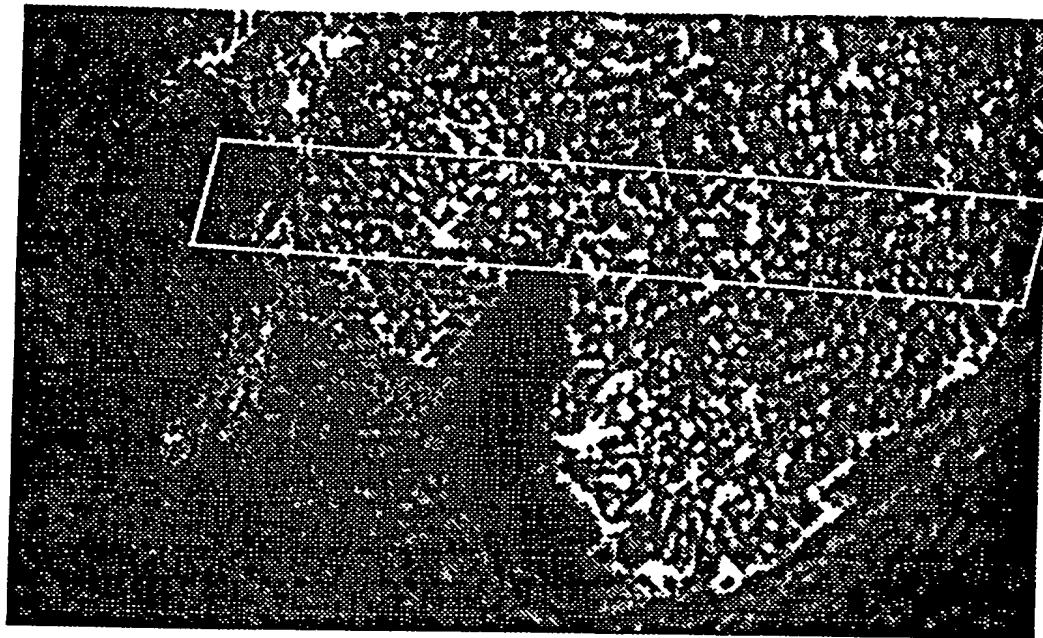
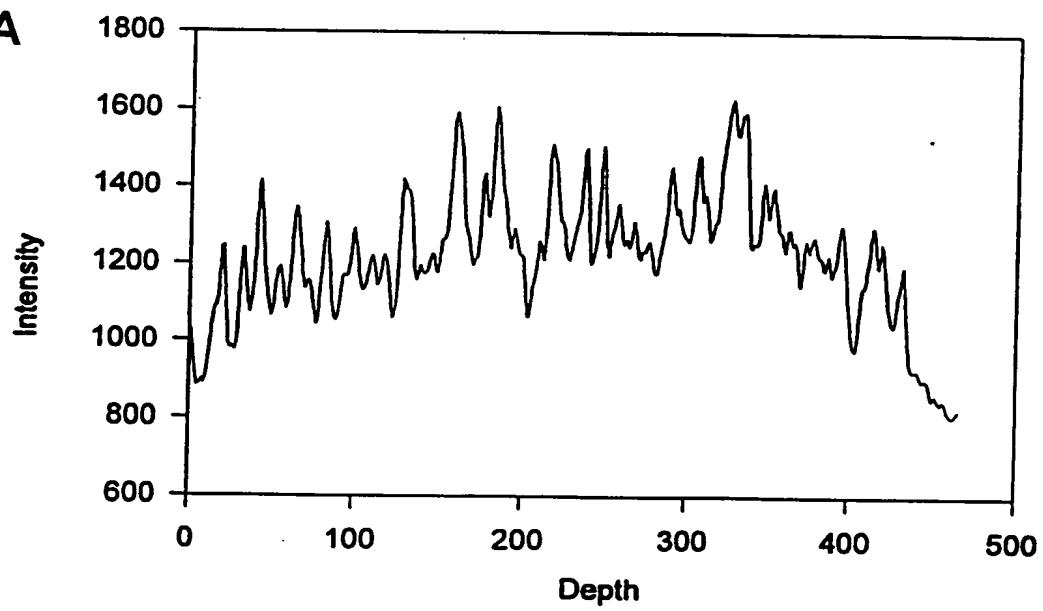
14 / 23

B**153-11****A**Figure 14

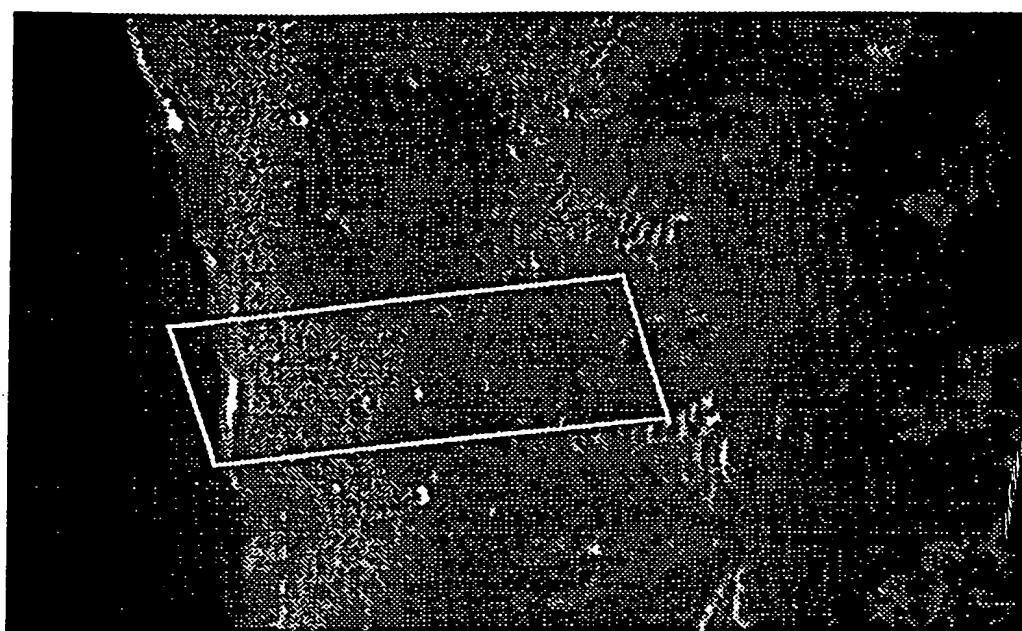
15 / 23

B**158-1****A****Figure 15**

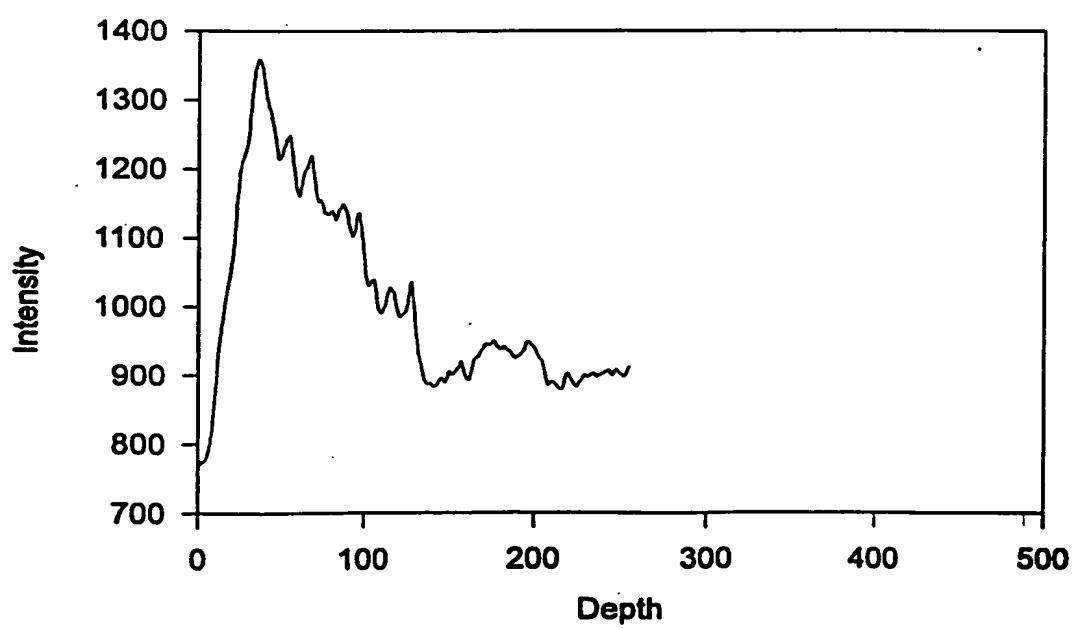
16 / 23

B**159-4****A****Figure 16**

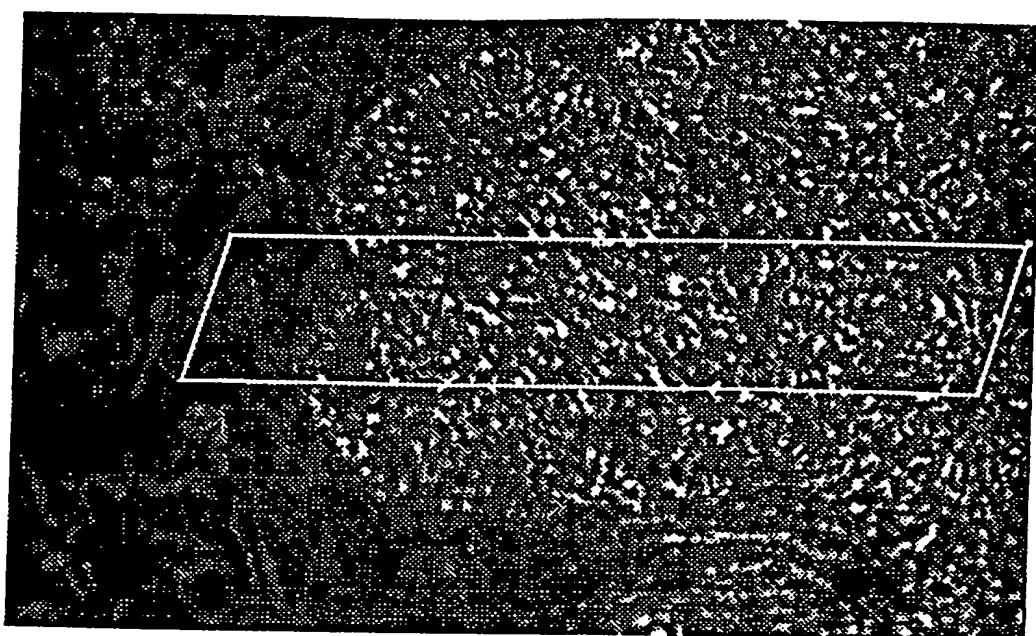
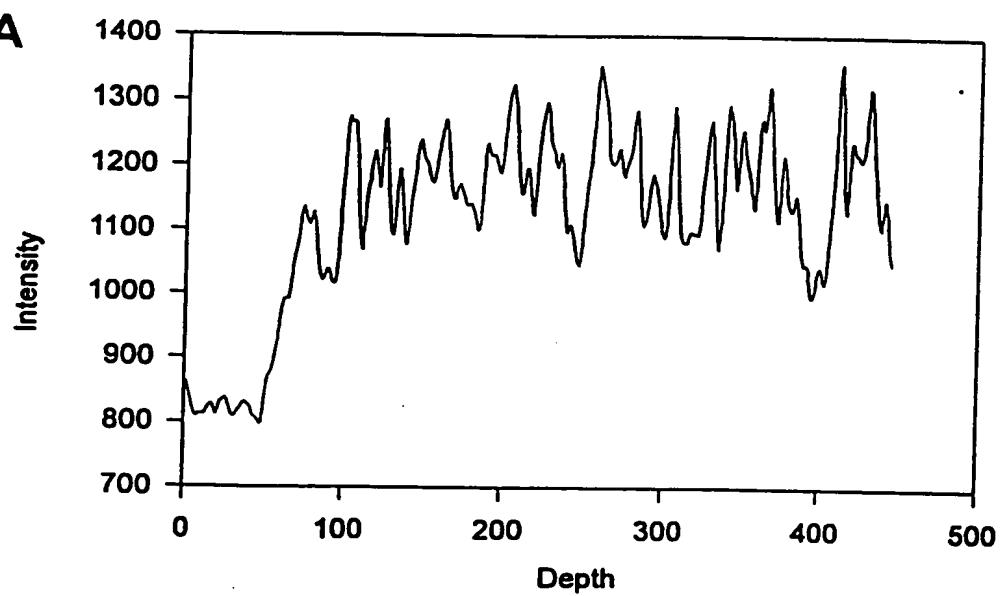
17 / 23

B**A**

160-1

Figure 17

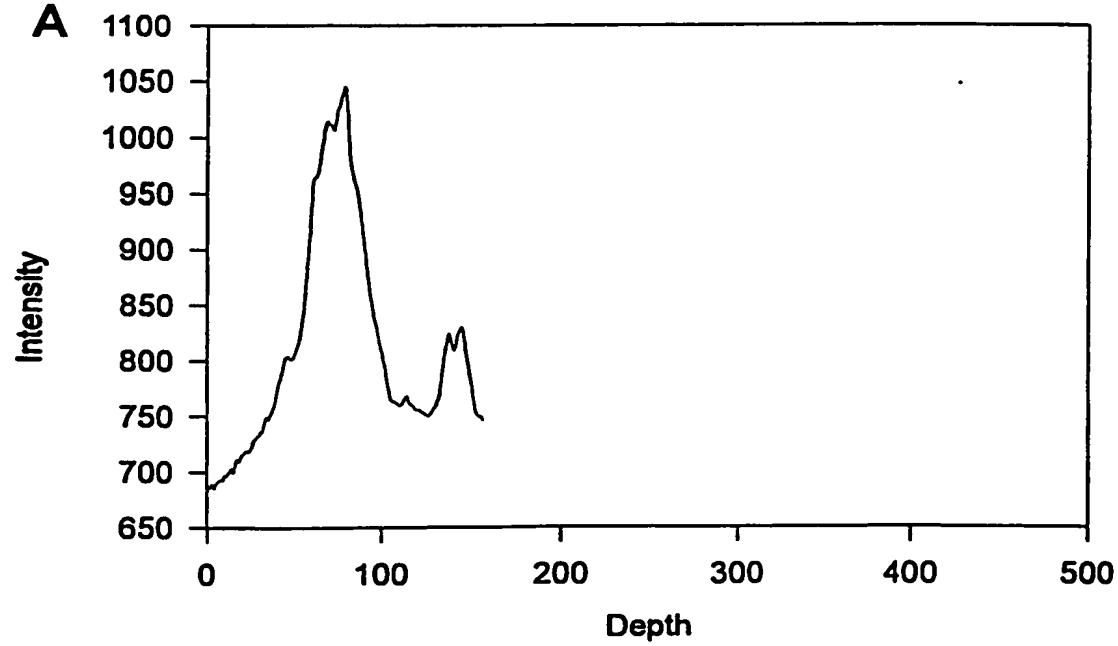
18 / 23

B**161-6****A****Figure 18**

19 / 23

B

162-1

A**Figure 19**

20 / 23

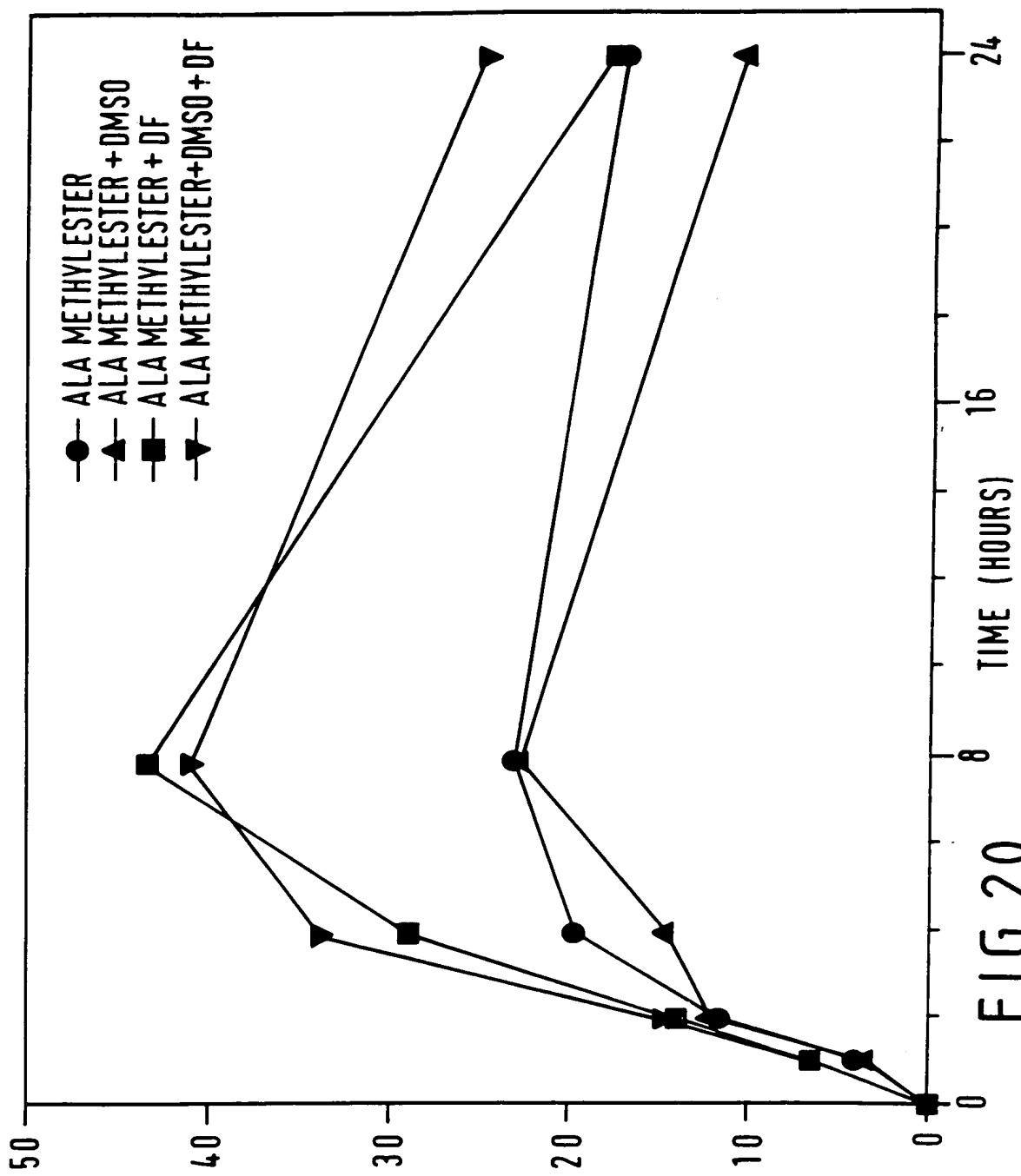


FIG. 20.

21 / 23

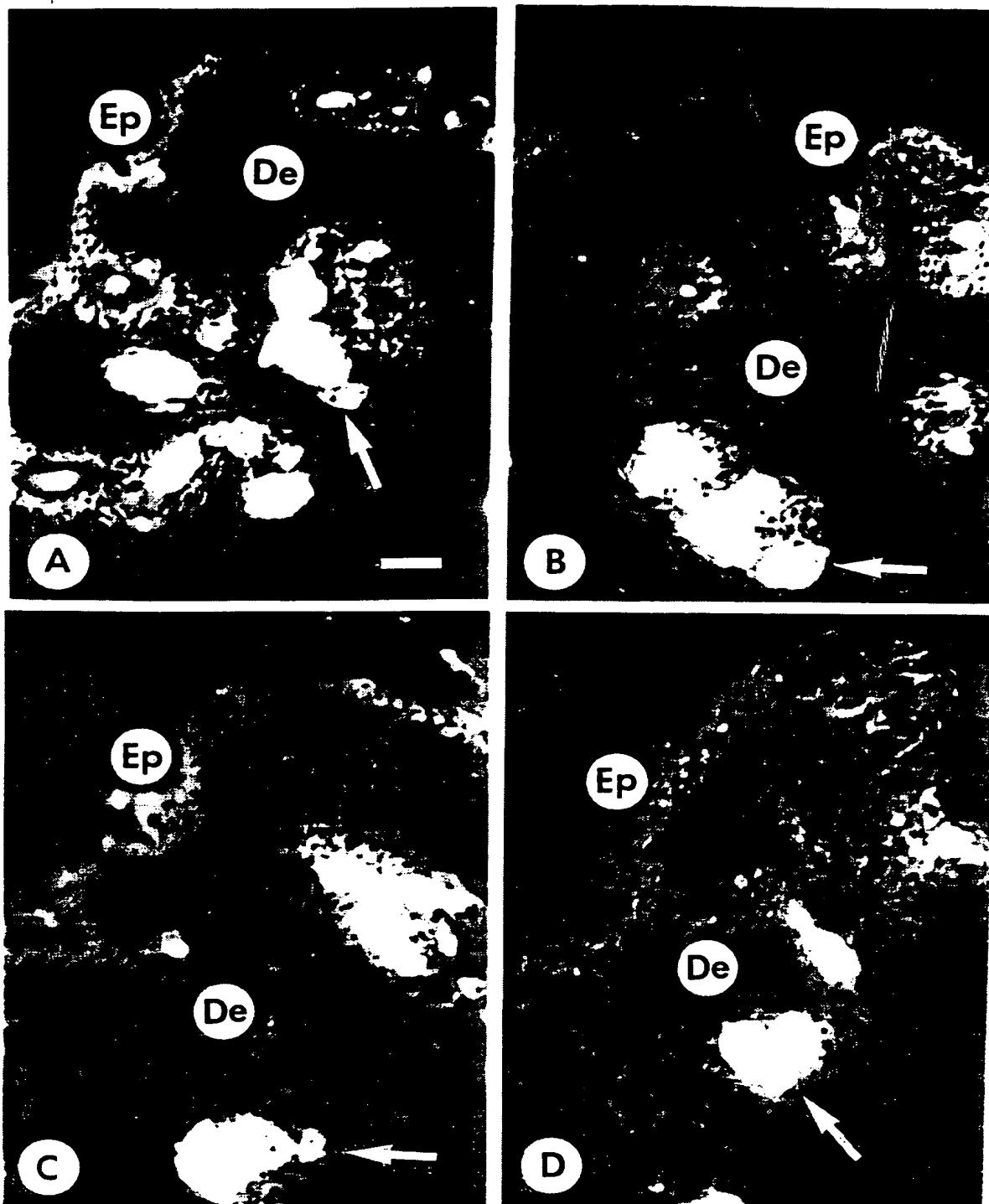
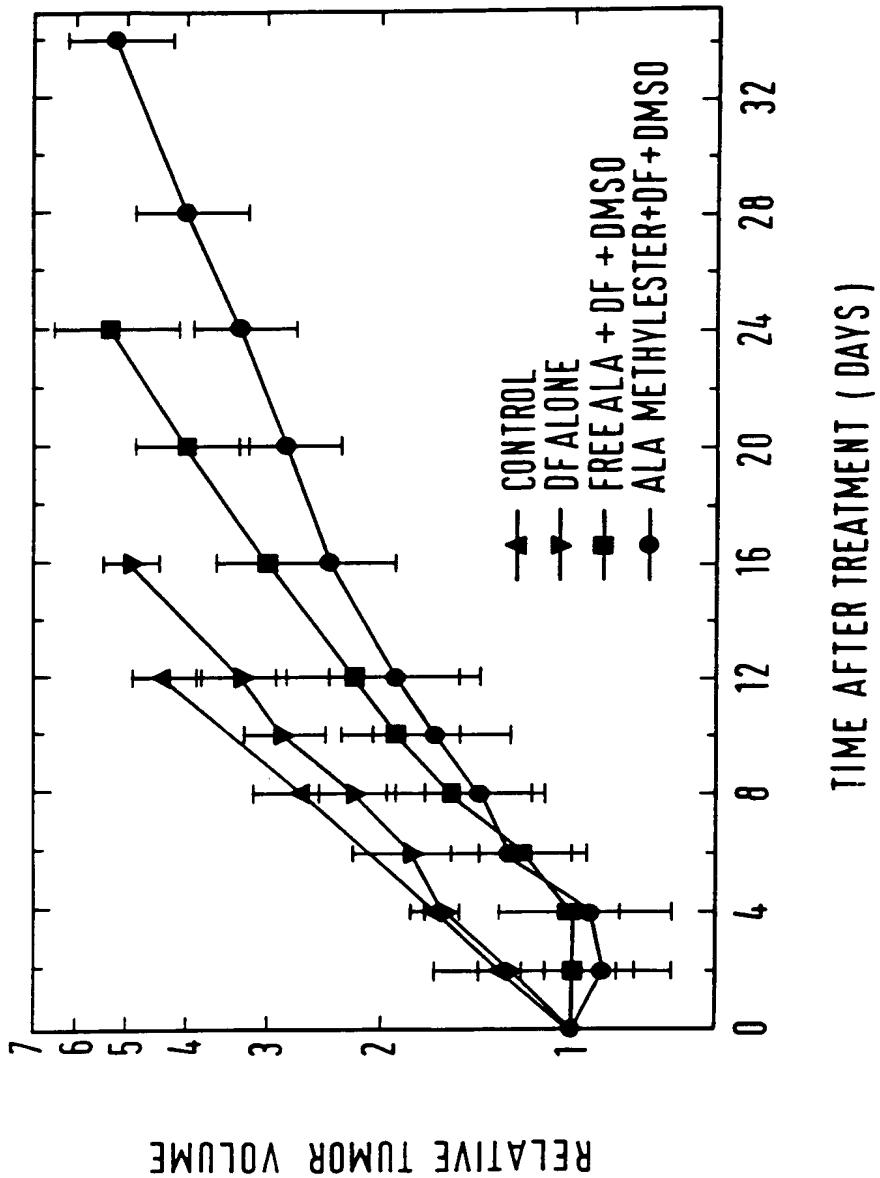


FIG. 21.

22/23

FIG. 22.



23 / 23

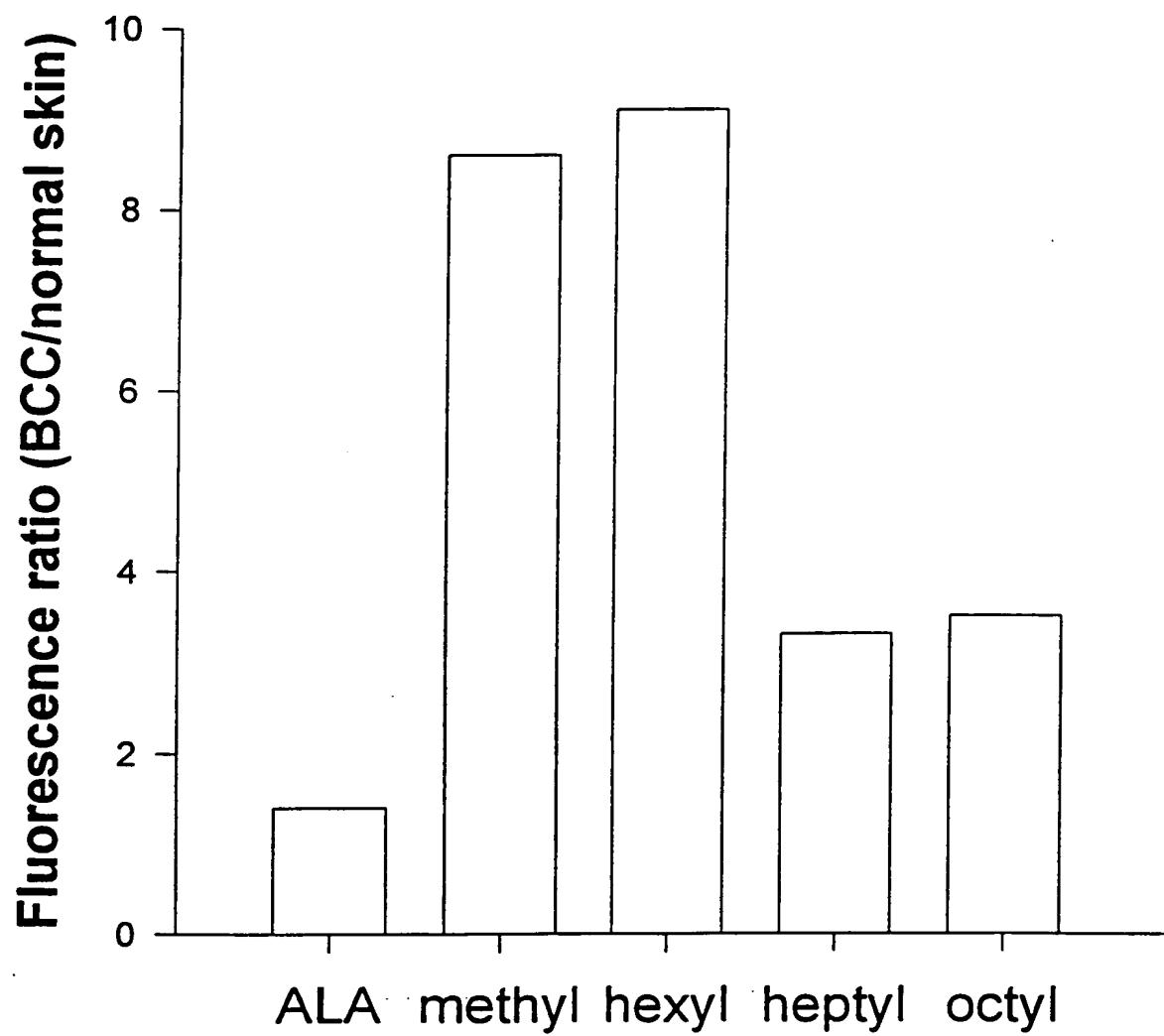


Figure 23

INTERNATIONAL SEARCH REPORT

...nal Application No

PC./GB 96/00553

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C07C229/22 C07C227/18 A61K31/195

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Database Xfire, entries 3060978, 5347132, 5499790, 5620924, 5633390, 5991317 and 6517740", BEILSTEIN XP002005286	1-6
X	---	
X	PATENT ABSTRACTS OF JAPAN vol. 016, no. 156 (C-0930), 16 April 1992 & JP,A,04 009360 (COSMO SOGO KENKYUSHO:KK;OTHERS: 01), 14 January 1992, see abstract	1-6
X	---	
X	EP,A,0 316 179 (TOKUYAMA SODA KK) 17 May 1989 see Table I-A, line 41 (p. 44)	1-6

	-/-	

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

1 Date of the actual completion of the international search

Date of mailing of the international search report

11 June 1996

28.05.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+ 31-70) 340-3016

Authorized officer

Janus, S

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 96/00553

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 058 077 (SZELKE M;HUDSON D SHARPE R) 8 April 1981 see page 8, line 60 ---	1-6
X	DE,A,24 11 382 (BOEHRINGER SOHN INGELHEIM) 18 September 1975 see p. 19, general formula XIV ---	1-3
A	WO,A,91 01727 (UNIV KINGSTON) 21 February 1991 cited in the application -----	7-19

INTERNATIONAL SEARCH REPORT

ational application No.

PCT/GB96/00553

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 14 and 15 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

na Application No

PCT/GB 96/00553

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0316179	17-05-89	JP-A- 1052778 AU-B- 2500588 DE-D- 3887275 DE-T- 3887275 JP-A- 2028154 JP-B- 7045502 US-A- 4882438 US-A- 4960678	28-02-89 11-05-89 03-03-94 05-05-94 30-01-90 17-05-95 21-11-89 02-10-90
GB-A-2058077	08-04-81	US-A- 4304715	08-12-81
DE-A-2411382	18-09-75	AT-B- 344332 AT-B- 348143 AT-B- 348144 AT-B- 348145 AT-B- 348146 AT-B- 348147 AT-B- 348148 AT-B- 348149 AU-B- 7894275 BE-A- 826440 BG-A- 25087 BG-A- 24813 BG-A- 24814 CA-A- 1049511 CH-A- 612425 CH-A- 620439 CH-A- 622014 CH-A- 622015 CH-A- 620440 CH-A- 622016 CH-A- 620681 CH-A- 622017 FR-A, B 2262984 GB-A- 1496418 JP-C- 1194779 JP-A- 50123675 JP-B- 58027275 NL-A- 7502724 SE-B- 421919	10-07-78 25-01-79 25-01-79 25-01-79 25-01-79 25-01-79 25-01-79 25-01-79 16-09-76 08-09-75 12-07-78 12-05-78 12-05-78 27-02-79 31-07-79 28-11-80 13-03-81 13-03-81 28-11-80 13-03-81 15-12-80 13-03-81 03-10-75 30-12-77 12-03-84 29-09-75 08-06-83 11-09-75 08-02-82

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C07C229/22 C07C227/18 A61K31/195

According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)
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X	PATENT ABSTRACTS OF JAPAN vol. 016, no. 156 (C-0930), 16 April 1992 & JP,A,04 009360 (COSMO SOGO KENKYUSHO:KK;OTHERS: 01), 14 January 1992, see abstract	1-6
X	EP,A,0 316 179 (TOKUYAMA SODA KK) 17 May 1989 see Table I-A, line 41 (p. 44)	1-6
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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

11 June 1996

Date of mailing of the international search report

28.05.96

Name and mailing address of the ISA

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 NL - 2280 HV Rijswijk
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl.
 Fax (+ 31-70) 340-3016

Authorized officer

Janus, S

INTERNATIONAL SEARCH REPORT

Inter.	Application No.
PCT/GB 96/00553	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 058 077 (SZELKE M;HUDSON D SHARPE R) 8 April 1981 see page 8, line 60 ---	1-6
X	DE,A,24 11 382 (BOEHRINGER SOHN INGELHEIM) 18 September 1975 see p. 19, general formula XIV ---	1-3
A	WO,A,91 01727 (UNIV KINGSTON) 21 February 1991 cited in the application -----	7-19

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/GB 96/00553

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0316179	17-05-89	JP-A-	1052778	28-02-89
		AU-B-	2500588	11-05-89
		DE-D-	3887275	03-03-94
		DE-T-	3887275	05-05-94
		JP-A-	2028154	30-01-90
		JP-B-	7045502	17-05-95
		US-A-	4882438	21-11-89
		US-A-	4960678	02-10-90
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GB-A-2058077	08-04-81	US-A-	4304715	08-12-81
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DE-A-2411382	18-09-75	AT-B-	344332	10-07-78
		AT-B-	348143	25-01-79
		AT-B-	348144	25-01-79
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		AT-B-	348146	25-01-79
		AT-B-	348147	25-01-79
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		BE-A-	826440	08-09-75
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		CH-A-	612425	31-07-79
		CH-A-	620439	28-11-80
		CH-A-	622014	13-03-81
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		CH-A-	620440	28-11-80
		CH-A-	622016	13-03-81
		CH-A-	620681	15-12-80
		CH-A-	622017	13-03-81
		FR-A,B	2262984	03-10-75
		GB-A-	1496418	30-12-77
		JP-C-	1194779	12-03-84
		JP-A-	50123675	29-09-75
		JP-B-	58027275	08-06-83
		NL-A-	7502724	11-09-75
		SE-B-	421919	08-02-82

INTERNATIONAL SEARCH REPORT

PCT/GB96/00553

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Although claims 14 and 15 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound.

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Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 96/00553

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
DE-A-2411382		SE-A-	7502592	10-09-75
		US-A-	4087532	02-05-78
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WO-A-9101727	21-02-91	US-A-	5079262	07-01-92
		AU-B-	624985	25-06-92
		AU-B-	6034390	11-03-91
		JP-T-	4500770	13-02-92
		NL-T-	9021172	01-07-91
		US-A-	5422093	06-06-95
		US-A-	5211938	18-05-93
		US-A-	5234940	10-08-93
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/00553

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
DE-A-2411382		SE-A-	7502592	10-09-75
		US-A-	4087532	02-05-78
-----	-----	-----	-----	-----
WO-A-9101727	21-02-91	US-A-	5079262	07-01-92
		AU-B-	624985	25-06-92
		AU-B-	6034390	11-03-91
		JP-T-	4500770	13-02-92
		NL-T-	9021172	01-07-91
		US-A-	5422093	06-06-95
		US-A-	5211938	18-05-93
		US-A-	5234940	10-08-93
-----	-----	-----	-----	-----

PATENT COOPERATION TREATY

PCT

REC'D	26 JUN 1997
WIPO	PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 27.42.62655/002	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 96/ 00553	International filing date (day/month/year) 08/03/1996	Priority date (day/month/year) 10/03/1995
International Patent Classification (IPC) or national classification and IPC C07C229/22		
Applicant PHOTOCURE AS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of 18 sheets.

3. This report contains indications and corresponding pages relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 09/10/1996	Date of completion of this report 26.06.97
Name and mailing address of the IPEA/ European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer  S. Janus Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/GB96/00553

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[] the international application as originally filed.

[x] the description, pages 2, 10, 12, 17-35_____, as originally filed,
pages _____, filed with the demand,
pages 1, 3-9, 9a, 11, 13-16_____, filed with the letter of 10/03/97,
pages _____, filed with the letter of _____,

[x] the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-18_____, filed with the letter of 10/03/97,
Nos. _____, filed with the letter of _____,

[] the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[] the description, pages _____.
[] the claims, Nos. _____.
[] the drawings, sheets/fig _____.

3. [] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/GB96/00553

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

- the entire international application,
 claims Nos. 13, 14 _____

because:

- the said international application, or the said claims Nos. 13, 14 _____ relate to the following subject matter which does not require an international preliminary examination (specify):

These claims are directed to methods of diagnostic and therapy performed on either humans or animals. Under the terms of R. 67.1 (iv), the International Preliminary Examination Authority is not required to carry out an examination on such claims.

For the assessment of the presently worded claims on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognise as industrially applicable claims to the use of a compound in a medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

- the description, claims or drawings (indicate particular elements below) or said claims Nos. _____ are so unclear that no meaningful opinion could be formed (specify):

- the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.
PCT/GB96/00553

[] no international search report has been established for said claims

Nos. _____

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/GB96/00553

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N) Claims 1-12, 15-18 _____ YES
 Claims _____ NO

Inventive Step (IS) Claims 6 _____ YES
 Claims 1-5, 7-12, 15-18 _____ NO

Industrial Applicability (IA) Claims 1-12, 15-18 _____ YES
 Claims _____ NO

2. CITATIONS AND EXPLANATIONS

1. WO-A-91/01727 (D6) discloses a method of detection and treatment of lesions by photochemotherapy; in this method, a precursor of protoporphyrin IX in the biosynthetic pathway for heme is administered so that protoporphyrin IX accumulates in the lesion, thereby inducing fluorescence (see p. 7, lines 10-18). More specifically, 5-aminolevulinic acid (ALA) is said to be preferred for this purpose (see p. 8, lines 1-6). However, the teaching of D6 is not limited to the use of said ALA, but rather includes that of all possible natural protoporphyrin IX precursors. In this light, the present subject-matter must be regarded as the provision of an alternative to the teachings of D6.

The solution provided by claim 1 consists of known compounds, namely possibly N-substituted alkyl esters of ALA. However, no medical indication of these compounds could be found in the available prior art, so that the subject-matter of claims 1-12 and 15-18 can be regarded as meeting the requirements of Art. 33.2 PCT.

2. The fact that the present compounds are unnatural precursors of protoporphyrin IX must be regarded as a priori obvious. Indeed, it is well known to the skilled person that esters can easily be hydrolysed *in vivo*.

Also the fact that, due to their increased lipophilicity, the esters penetrate the skin more easily and are therefore more effective must be regarded as obvious, the precise degree of improvement being a mere bonus.

However, the fact that the esters are not, as free ALA is, transported by the blood to other tissues must be regarded as a priori unexpected and allows therefore the acknowledgement of inventive step for the compounds for which this effect has been demonstrated.

Indeed, this effect has only been demonstrated for three compounds, all belonging to the class of unsubstituted alkyl esters of N-unsubstituted ALA. Whereas these compounds can be regarded as adequate solution of the technical problem faced by the invention, there is no indication that N-substituted compounds are effective solutions of the problem as well.

In addition, it appears that the substitution of the nitrogen of ALA is an equally substantial structural modification as the esterification. Therefore, the effect of this modification must also be demonstrated for N-substituted compounds. It is interesting to note that no N-substituted compounds has in fact been prepared in the examples of the application. It cannot therefore be said that it would not be fair to the applicant to restrict the scope of the claims to N-unsubstituted compounds. If the applicant believes, as implicitly stated in the letter of 10/03/97 (see p. 2, penultimate

paragraph), that N-substituted compounds are indeed active, then he is invited to support his opinion by adequate data.

In the absence of such data, no inventive step can possibly be acknowledged for this subject-matter; claims 1-5, 7-12 and 15-18 do not therefore meet the requirements of Art. 33.3 PCT.

Claim 6, on the other hand, because it relates to N-unsubstituted derivatives, is regarded as meeting said requirements.

3. Industrial applicability, on the other hand, can be acknowledged for the whole of the examined subject-matter (Art. 33.4 PCT).

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:
DZIEGLEWSKA, Hanna, Eva
FRANK B. DEHN & CO.
179 Queen Victoria Street
London EC4V 4EL
GRANDE BRETAGNE

<div style="border: 1px solid black; padding: 5px; display: inline-block;"> FILE 62655/002 RECEIVED ANSO </div>	<p style="text-align: center;">NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT</p> <p style="text-align: center;">(PCT Rule 71.1)</p> <p style="text-align: right;">Date of mailing (day/month/year) 24.06.97</p>		
<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 2px;">Applicant's or agent's file reference 27.42.62655/002</td> <td style="width: 50%; padding: 2px; text-align: center;">IMPORTANT NOTIFICATION</td> </tr> </table>		Applicant's or agent's file reference 27.42.62655/002	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 27.42.62655/002	IMPORTANT NOTIFICATION		
International application No. PCT/GB 96/00553	International filing date (day/month/year) 08/03/1996	Priority date (day/month/year) 10/03/1995	
<p>Applicant PHOTOCURE AS et al.</p>			

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

<p>Name and mailing address of the IPEA/</p> <p>European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465</p>	<p>Authorized officer</p> <p><i>H. Hinkel 8</i> W. Hinkel</p>
<p>Telephone No. 8632</p>	

PATENT COOPERATION TREATY

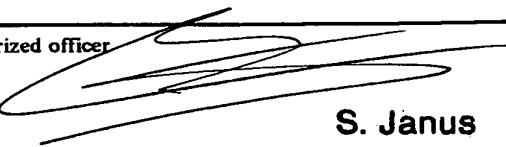
PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 27.42.62655/002	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 96/ 00553	International filing date (<i>day/month/year</i>) 08/03/1996	Priority date (<i>day/month/year</i>) 10/03/1995
International Patent Classification (IPC) or national classification and IPC C07C229/22		
Applicant PHOTOCURE AS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
- This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).
These annexes consists of a total of 18 sheets.
3. This report contains indications and corresponding pages relating to the following items:
- I Basis of the report
 - II Priority
 - III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV Lack of unity of invention
 - V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI Certain documents cited
 - VII Certain defects in the international application
 - VIII Certain observations on the international application

Date of submission of the demand 09/10/1996	Date of completion of this report 24.05.97
Name and mailing address of the IPEA/  European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer  S. Janus Telephone No. (09/04/1997)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/GB96/00553

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[] the international application as originally filed.

[x] the description, pages 2, 10, 12, 17-35_____, as originally filed,
pages _____, filed with the demand,
pages 1, 3-9, 9a, 11, 13-16_____, filed with the letter of 10/03/97,
pages _____, filed with the letter of _____,

[x] the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-18_____, filed with the letter of 10/03/97,
Nos. _____, filed with the letter of _____,

[] the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[] the description, pages _____.
[] the claims, Nos. _____.
[] the drawings, sheets/fig _____.

3. [] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/GB96/00553

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

- the entire international application,
- claims Nos. 13, 14 _____

because:

- the said international application, or the said claims Nos. 13, 14 _____ relate to the following subject matter which does not require an international preliminary examination (specify):

These claims are directed to methods of diagnostic and therapy performed on either humans or animals. Under the terms of R. 67.1 (iv), the International Preliminary Examination Authority is not required to carry out an examination on such claims.

For the assessment of the presently worded claims on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognise as industrially applicable claims to the use of a compound in a medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

- the description, claims or drawings (indicate particular elements below) or said claims Nos. _____ are so unclear that no meaningful opinion could be formed (specify):

- the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/GB96/00553

[] no international search report has been established for said claims

Nos. _____

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/GB96/00553

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**1. STATEMENT**

Novelty (N) Claims 1-12, 15-18 _____ YES
 Claims _____ NO

Inventive Step (IS) Claims 6 _____ YES
 Claims 1-5, 7-12, 15-18 _____ NO

Industrial Applicability (IA) Claims 1-12, 15-18 _____ YES
 Claims _____ NO

2. CITATIONS AND EXPLANATIONS

1. WO-A-91/01727 (D6) discloses a method of detection and treatment of lesions by photochemotherapy; in this method, a precursor of protoporphyrin IX in the biosynthetic pathway for heme is administered so that protoporphyrin IX accumulates in the lesion, thereby inducing fluorescence (see p. 7, lines 10-18). More specifically, 5-aminolevulinic acid (ALA) is said to be preferred for this purpose (see p. 8, lines 1-6). However, the teaching of D6 is not limited to the use of said ALA, but rather includes that of all possible natural protoporphyrin IX precursors. In this light, the present subject-matter must be regarded as the provision of an alternative to the teachings of D6.

The solution provided by claim 1 consists of known compounds, namely possibly N-substituted alkyl esters of ALA. However, no medical indication of these compounds could be found in the available prior art, so that the subject-matter of claims 1-12 and 15-18 can be regarded as meeting the requirements of Art. 33.2 PCT.

2. The fact that the present compounds are unnatural precursors of protoporphyrin IX must be regarded as a priori obvious. Indeed, it is well known to the skilled person that esters can easily be hydrolysed *in vivo*.

Also the fact that, due to their increased lipophilicity, the esters penetrate the skin more easily and are therefore more effective must be regarded as obvious, the precise degree of improvement being a mere bonus.

However, the fact that the esters are not, as free ALA is, transported by the blood to other tissues must be regarded as a priori unexpected and allows therefore the acknowledgement of inventive step for the compounds for which this effect has been demonstrated.

Indeed, this effect has only been demonstrated for three compounds, all belonging to the class of unsubstituted alkyl esters of N-unsubstituted ALA. Whereas these compounds can be regarded as adequate solution of the technical problem faced by the invention, there is no indication that N-substituted compounds are effective solutions of the problem as well.

In addition, it appears that the substitution of the nitrogen of ALA is an equally substantial structural modification as the esterification. Therefore, the effect of this modification must also be demonstrated for N-substituted compounds. It is interesting to note that no N-substituted compounds has in fact been prepared in the examples of the application. It cannot therefore be said that it would not be fair to the applicant to restrict the scope of the claims to N-unsubstituted compounds. If the applicant believes, as implicitly stated in the letter of 10/03/97 (see p. 2, penultimate

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.

PCT/GB96/00553

paragraph), that N-substituted compounds are indeed active, then he is invited to support his opinion by adequate data.

In the absence of such data, no inventive step can possibly be acknowledged for this subject-matter; claims 1-5, 7-12 and 15-18 do not therefore meet the requirements of Art. 33.3 PCT.

Claim 6, on the other hand, because it relates to N-unsubstituted derivatives, is regarded as meeting said requirements.

3. Industrial applicability, on the other hand, can be acknowledged for the whole of the examined subject-matter (Art. 33.4 PCT).

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

FRANK B. DEHN & CO.
Attn. DZIEGLEWSKA, Hanna-Eva
179 Queen Victoria Street
LONDON EC4V 4EL
UNITED KINGDOM

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

FILE 62655/002

- 3 JUL 1996

RECEIVED

ANSO

Date of mailing
(day/month/year) 23.06.96

Applicant's or agent's file reference
27.42.62655/002

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/GB96/00553

International filing date
(day/month/year) 08/03/96

Applicant

THE NORWEGIAN RADIUM HOSPITAL RESEARCH et al.

1. The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? To the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. With regard to the protest against payment of (an) additional fee(s) under Rule 40.2; the applicant is notified that:

the protest together with the decision thereon has been transmitted to the International Bureau together with the applicants's request to forward the texts of both the protest and the decision thereon to the designated Office.

no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Further action(s): The applicant is reminded of the following:

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

DEAD DATES NOTED
Aug 28/96

Name and mailing address of the International Searching Authority

European Patent Office, P.B. 5818 Patentiaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

M. PFIS

NOTES TO FORM PCT/ISA/220

These notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty and of the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

The claims only.

The description and the drawings may only be amended during international preliminary examination under Chapter II.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confounded with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

NOTES TO FORM PCT/ISA/220 (continued)

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers;
Claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 TO 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings which cannot be amended under Article 19(1).

The statement will be published with the international application and the amended claims.

The statement should be brief, it should not exceed 500 words if in English or if translated into English.

It should not be confounded with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It should not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

In what language?

The amendments must be made in the language in which the international application is published. The letter and any statement accompanying the amendments must be in the same language as the international application if that language is English or French; otherwise, it must be in English or French, at the choice of the applicant.

Consequence if a demand for international preliminary examination has already been filed?

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase?

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 27.42.62655/002	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/GB96/00553	International filing date (<i>day/month/year</i>) 08/03/96	(Earliest) Priority Date (<i>day/month/year</i>) 10/03/95
Applicant		
THE NORWEGIAN RADIUM HOSPITAL RESEARCH et al.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.
 It is also accompanied by a copy of each prior art document cited in this report.

1. Certain claims were found unsearchable (see Box I).
2. Unity of invention is lacking (see Box II).
3. The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing
 - filed with the international application.
 - furnished by the applicant separately from the international application,
 - but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
 - Transcribed by this Authority

4. With regard to the title, the text is approved as submitted by the applicant
 the text has been established by this Authority to read as follows:

**ESTERS OF 5-AMINOLEVULINIC ACID AS PHOTOSENSITIZING AGENTS IN
PHOTOCHEMOTHERAPY**

5. With regard to the abstract,
 - the text is approved as submitted by the applicant
 - the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the drawings to be published with the abstract is:
 - as suggested by the applicant
 - because the applicant failed to suggest a figure.
 - because this figure better characterizes the invention.

None of the figures.

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 14 and 15 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 96/00553

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C07C229/22 C07C227/18 A61K31/195

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Database Xfire, entries 3060978, 5347132, 5499790, 5620924, 5633390, 5991317 and 6517740" , BEILSTEIN XP002005286	1-6
X	--- PATENT ABSTRACTS OF JAPAN vol. 016, no. 156 (C-0930), 16 April 1992 & JP,A,04 009360 (COSMO SOGO KENKYUSHO:KK;OTHERS: 01), 14 January 1992, see abstract	1-6
X	--- EP,A,0 316 179 (TOKUYAMA SODA KK) 17 May 1989 see Table I-A, line 41 (p. 44) ---	1-6
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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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